

## EFFECT OF GUM ARABIC EDIBLE COATING ON WEIGHT LOSS, FIRMNESS AND SENSORY CHARACTERISTICS OF CUCUMBER (*CUCUMIS SATIVUS* L.) FRUIT DURING STORAGE

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### Abstract

The effect of application of gum arabic edible coating on weight loss, firmness and sensory characteristics was investigated for cucumber fruits. Cucumber was coated with gum arabic at different concentration (5, 10, 15 and 20%) and stored at 10 and 25°C for up to 16 days. Gum coating significantly reduced weight loss of the fruits at both storage temperatures. The firmness of the control fruits significantly ( $p \leq 0.05$ ) decreased with the storage time at both 10 and 25°C. The application of gum edible coating delayed softening of cucumber fruit during 16 days of storage at 10 and 25°C. Sensory characteristics of cucumber such as color, taste, tenderness, appearance and overall acceptability of coated (5-20%) cucumbers were much better preserved while storing at 10 and 25°C for 16 days.

### Introduction

Cucumber (*Cucumis sativus* L.) is an important plant in the world, fruit of which can be used in as fresh or cooked in many countries. Cucumbers quality is highly reduced after harvesting because of the loss of water, shriveling and also yellowing and its shelf-life in the market is limited to 2~3 days. One of the main goals of postharvest technology in vegetables is to delay senescence symptoms. Changes in peel color are natural in horticultural commodities and are part of the ripening and the natural senescence process (Funamoto *et al.*, 2002). Changes in color can be accelerated by stress, such as chilling injury, ethylene exposure and decay but can also occur naturally during storage. Unlike other fruits, such as tomato and bananas that need to undergo ripening to reach commercial maturity, this process is detrimental to fruit quality in cucumber since it reaches commercial maturity at a physiologically immature stage. The rate of green color has been associated with keeping quality of cucumbers (Schoutes *et al.*, 2002). The senescence symptoms of cucumber (*Cucumis sativus* L.) could be reduced by packaging and cold storage. However, cucumber is a chilling sensitive commodity and thus should not be stored long-term at temperatures below 7-10°C (Snowdon, 1991). If cucumbers are stored at lower temperatures, chilling injury may develop and it is generally followed by an increased tendency to decay, particularly when the temperature is raised (DeEll, *et al.*, 2000). The application of edible coatings can improve the physical strength of food products, reduce particle clustering, and improve visual and tactile features on product surfaces (Cuq, *et al.*, 1995; Cisneros-Zevallos, *et al.*, 1997). The coatings can also protect food products from moisture migration, microbial growth on the surface, light-induced chemical changes, oxidation of nutrients, etc. Edible coatings can act as barriers against oils, gases, or vapors and as carriers of active substances such as antioxidants, antimicrobials, colors and flavors (Gennadios & Weller, 1990; Krochta & De Mulder-Johnston 1997; Miller, *et al.*, 1998). These functions

enhance the quality of food products, resulting in shelf-life extension and safety improvement. Further, edible coatings can be utilized as active films when applied to modify the atmosphere of food surface conditions (Cuq *et al.*, 1995; Guilbert & Gontard, 2005). Gums have been used in foods due to their different beneficial characteristics (Ghafoor *et al.*, 2008). Gum arabic or gum acacia is dried, gummy exudates from the stems or branches of *Acacia* species. It is the least viscous and most soluble of the hydrocolloids (Nisperos-Carriedo, 1994), and is used extensively in the industrial sector because of its emulsification, film forming and encapsulation properties (Motlagh *et al.*, 2006). More than one half of the world's supply is used in confectionary to retard sugar crystallization and to thicken candies, jellies, glazes and chewing gums (Motlagh *et al.*, 2006).

The objective of our present research was to investigate the effect of gum Arabic coating on weight loss, firmness and sensory characteristics of cucumber during storage at refrigeration and room temperatures.

### Materials and Methods

**Plant materials and treatments:** Fresh and mature cucumber (*Cucumis sativus* L. var. Rami) was obtained from a demonstration farm, Riyadh, Saudi Arabia and treated on the same day. Gum Arabic powder (Food Grade) was obtained from Khartoum Gum Arabic Processing Company (GAPC), Khartoum, Sudan. Good quality cucumber fruits (250), devoid of any defects, were selected with homogenous size, color and maturity and randomly grouped into five lots (120 fruit each). Then the fruits in each lot were washed with a solution of sodium hypochlorite (0.05%) for 3 min and air dried at ambient temperature. To prepare gum arabic coating solutions at 5, 10, 15, 20% (w/v) concentrations, 5, 10, 15 and 20g of powder was dissolved in 100ml purified water, respectively. The solutions were stirred with low heat (40°C) for 60 min on a hot plate magnetic stirrer (Wiess Gallenkamp, Leicestershire, UK), then filtered to remove any un-dissolved impurities using a vacuum flask. After

cooling to 20°C, glycerol monostearate (1.0%) (Sigma-Aldrich, St. Louis, MO, USA) was added as a plasticizer to improve the strength and flexibility of the coating solutions. The cucumber fruits of each lot were dipped in each concentration of gum arabic coating solution or distilled water (control lot) for 3 min. The coating solution was applied uniformly on the whole surface. Then the fruits were taken out and allowed to dry at ambient temperature. A dry layer with plastic texture appeared and the general appearance were used as criteria to determine the end of drying. Gum Arabic films were prepared according to Miranda *et al.*, (2004) method and the thickness was measured with a micrometer (Mitutoyo, Kanagawa, Japan) to the nearest 0.01 mm at four locations; average values were (5%:  $0.011 \pm 0.001$  mm; 10%:  $0.013 \pm 0.001$  mm; 15%:  $0.016 \pm 0.002$  mm; 20%  $0.021 \pm 0.001$  mm). After treatment, cucumber fruits were packed in cardboard boxes (60 fruits per box) and stored at 10 or 25  $\pm$ 1°C and 80-90 RH for 16 days. The data were recorded before treatment (day 0) and at 4 days intervals up to 16 days.

**Firmness measurement:** On each sampling day, 3 cucumber fruits from each treatment were analyzed for firmness using Texture Analyzer (Model: TA HDi, Stable Micro Systems, HD3128, Surrey, England) equipped with a 7.9 mm cylindrical probe with a flat surface interfaced with a computer. The acquisition rate was 200 PPS and height was 100mm. The distance and temperature were 5mm and 25°C, respectively. Force (N) readings were recorded at 2% constant deformation on the circumference of each of 3 fruits from coated and uncoated groups.

**Weight loss percentage:** Cucumber samples (3 fruits) were weighed at day 0 and at the end of each storage interval. The difference between initial and final fruit weight was considered as total weight loss during that storage interval and calculated as percentages on a fresh weight basis by the standard Anon., (2000) method.

**Sensory evaluation:** The sensory tests were conducted using 5-hedonic scale, by a semi-trained panel. Ten judges were selected who had successfully passed standardized tests for olfactory and taste sensitivities as well as verbal abilities and creativity. The panelists were given a hedonic questionnaire to test color, taste, tenderness, appearance and overall acceptability of coded samples of cucumber as a control, and after treatment with 5%, 10%, 15% and 20% gum Arabic at each interval. They were scored on a scale of 1–5 (1 = poor, 2 = fair, 3 = good, 4 = very good and 5 = excellent).

**Statistical analysis:** Each determination was carried out on at least 3 separate samples and analyzed in triplicate on dry weight basis; the figures were then averaged. Data were assessed by the analysis of variance (ANOVA) as described by Snedecor and Cochran (1987). Comparisons of means for treatments were made using Duncan's multiple range tests. Significance was accepted at  $p \leq 0.05$ .

## Results and Discussion

**Effect of gum coating on weight loss and firmness of cucumber during storage:** Fig. 1 shows the effect of gum Arabic coating on weight loss of cucumber fruits stored at 10 and 25°C for different period of time (0-16 days). Weight loss of the control fruits significantly ( $p \leq 0.05$ ) increased with storage time and reached 8 and 11% at day 16 at 10°C (Fig. 1a) and 25°C (Fig. 1b), respectively. Fruits coated with Arabic gum had less weight loss during storage than the control, and weight loss increased gradually during the storage period. The results obtained indicated that gum significantly ( $p \leq 0.05$ ) reduced weight loss and acts as barrier against water loss. The basic mechanism of weight loss from fresh fruit and vegetables is by vapor pressure at different locations (Yaman & Bayoindirli, 2002), although respiration also causes a weight reduction (Pan & Bhowmilk, 1992). This reduction in weight loss was probably due to the effects of the coating as a semi-permeable barrier against O<sub>2</sub>, CO<sub>2</sub>, moisture and solute movement, thereby reducing respiration, water loss and oxidation reaction rates (Baldwin *et al.*, 1999; Park, 1999). The results are in agreement with the findings of Srinivasa *et al.*, (2006) for tomato and bell pepper packaged in cartons covered with either eco-friendly chitosan film or synthetic petroleum-based low-density polyethylene (LDPE) film who reported that chitosan and LDPE films extended the storage life of both tomato and bell pepper through reduction in water loss and a modification of the internal atmosphere. Variation in weight loss for gum Arabic concentrations was observed compared to control samples which could be explained by the difference in thickness of coatings. Weight loss at 20% gum was lower even at the end of storage period. However, Park *et al.*, (1993) reported that tomato fruit coated too thickly with a corn-zein film resulted in O<sub>2</sub> concentrations which were too low and excessive CO<sub>2</sub> concentrations, resulting in ethanol production. Further they reported that the primary reason for increased weight loss of thickly coated tomatoes might be the generation of heat and production of end-products from anaerobic fermentation.

The firmness of the control fruits significantly ( $p \leq 0.05$ ) decreased with storage time and reached 165 and 155 N at day 16 at 10°C (Fig. 2a) and 25°C (Fig. 2b), respectively. Fruits coated with Arabic gum had significantly ( $p \leq 0.05$ ) higher firmness values during storage than the control and the fruit firmness decreased gradually during the storage period. The results obtained indicated that gum significantly ( $p \leq 0.05$ ) retained the firmness of the fruits and acted as a barrier against nutrients and water loss. At the end of storage (16 days), control fruit clearly showed the lowest firmness. The maximum firmness was maintained by the 20% gum Arabic treated cucumber fruit until day 16. Softening of fruit is due to deterioration in the cell structure, cell wall composition and intracellular materials (Seymour *et al.*, 1993) and is a biochemical process involving the hydrolysis of pectin and starch by enzymes e.g. wall hydrolases. As the process of fruit ripening progresses, depolymerisation or shortening of chain length of pectin

substances occurs with an increase in pectinesterase and polygalacturonase activities (Yaman & Bayoindirli, 2002). Low levels of  $O_2$  and high levels of  $CO_2$  limit the activities of these enzymes and allow retention of the firmness during storage (Salunkhe *et al.*, 1991). In agreement with these findings, Park *et al.*, (1994) reported that respiration and  $O_2$  consumption of corn-zein coated

tomatoes were lower than for non-coated tomatoes. Reduction in respiration rates of coated cucumber could be responsible for delaying softening which resulted in retention of firmness during storage. Similarly, Tanada-Palmu and Grosso (2005) reported that refrigerated strawberries coated with wheat gluten-based films retained their firmness better than control fruit.

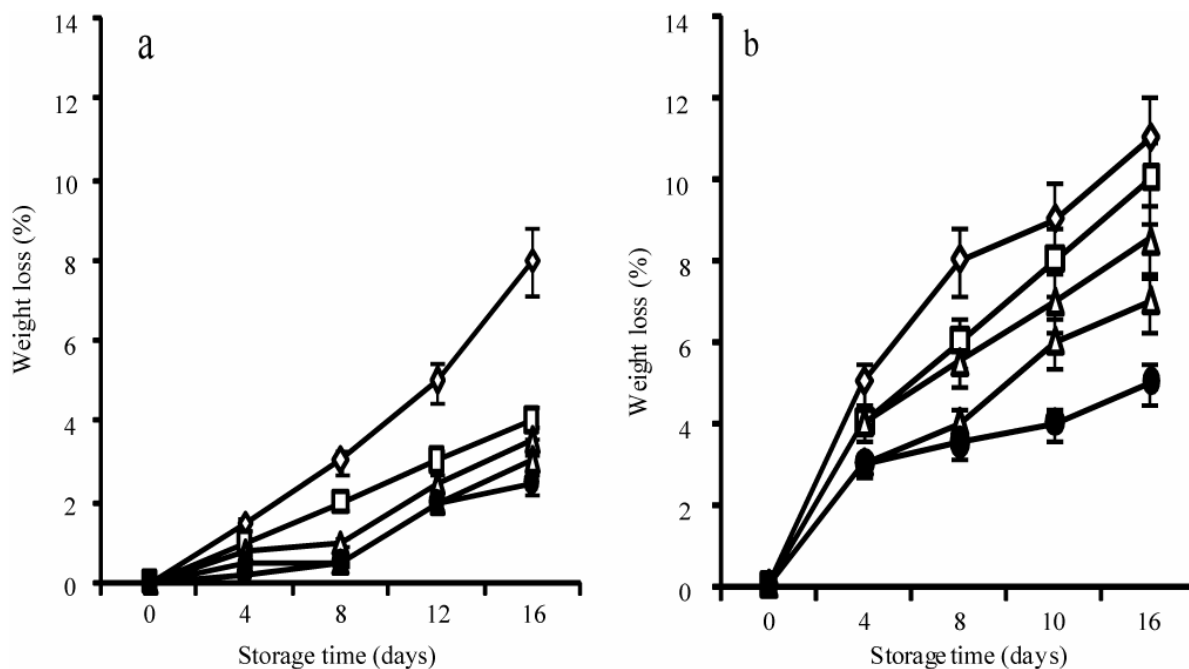


Fig. 1. Effect of coating of cucumber with gum Arabic at different concentration on weight loss during storage at 10°C (a) and 25°C (b). Control (◇), 5% (□), 10% (△), 15% (▲), 20% (●).

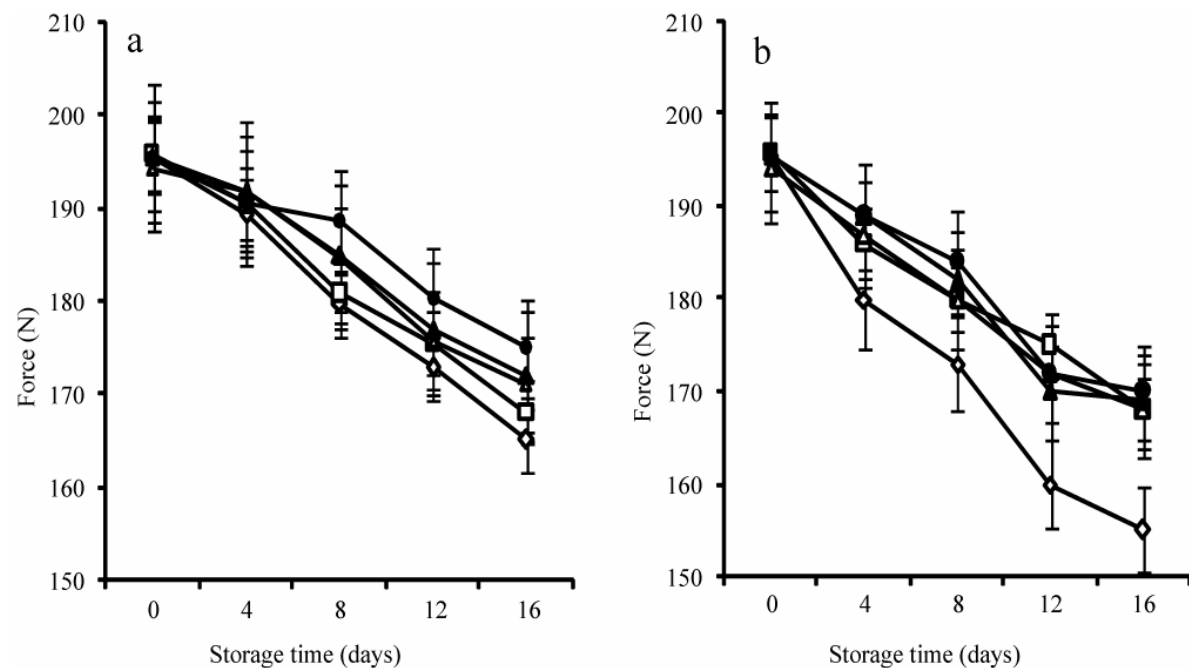


Fig. 2. Effect of coating of cucumber with gum Arabic at different concentration on firmness during storage at 10°C (a) and 25°C (b). Control (◇), 5% (□), 10% (△), 15% (▲), 20% (●).

**Sensory evaluation of treated and untreated cucumber during storage:** Furthermore, in order to confirm the role of gum Arabic as a keeping quality agent, sensory evaluation was carried out by 10 semi-trained panelists and the results are shown in Table 1. Coded samples were introduced to panelists to evaluate surface color, taste, tenderness, appearance and overall acceptability of coated and uncoated fruits at each interval. Sensory evaluation of coated and uncoated fruit at the end of the storage period revealed significant ( $p \leq 0.05$ ) differences in color, taste, tenderness, appearance and overall acceptability. The color acceptability was gradually decreased with the storage time for the control and treated fruits at 10 and 25°C. The acceptability of the color significantly ( $p \leq 0.05$ ) decreased at day 16 especially for the control fruits stored at 25°C. However, coating with gum Arabic (20%) retained the brightness of the fruits color as indicated by sensory data. The results obtained indicated that gum coating of cucumber fruits retained the brightness and green color of the fruits. All other sensory attributes such as taste, tenderness, appearance and overall acceptability followed the trend similar that obtained for the color. The 20% gum Arabic coated fruits had the highest scores in most parameters after 16 days of storage. Compared to 20% gum Arabic coated fruits, control fruit and fruit treated with 5% gum had lower scores for the quality attributes. The results obtained suggest that gum Arabic

up to 20% can be used successfully as an edible coating for prolonging the shelf-life and preserving cucumber fruit quality during storage at 10 and 25°C. Similar results were observed by El-Anany *et al.*, (2009) when they treated 'Anna' apples with gum Arabic coating. It has been reported that fruits and vegetables are severely stressed after harvest due to a reduction in the sources of energy, nutrients, hormones and water, and also this leads to a rapid initiation of senescence (King & Morris, 1994). In the case of cucumbers, the main symptoms of senescence are yellowing and shriveling due to water loss and these non conformities were observed during evaluation of non coated cucumbers for firmness and sensory attributes in our study. Gum Arabic coating might reduced the loss in energy, nutrients, hormones and water by acting as a barrier and therefore prevented loss of weight and delayed the initiation of senescence which decreases the firmness and impairs the sensory attributes of cucumber fruit.

We can infer based on this study that cucumber fruits coated with gum Arabic (<20% gum solution) showed a significant delay in the loss of weight and firmness up to 16 days at 10 and 25°C of storage temperatures. In addition, sensory evaluation for taste, color, tenderness, appearance and over all acceptability showed that gum Arabic coating maintained the overall quality of the cucumber during storage.

**Table 1. Effect of gum arabic coating on sensory evaluation of cucumber during storage at different period of time and temperature.**

Gum concentration (%)	Storage period (days)														
	Color					Taste					Tenderness				
	0	4	8	12	16	0	4	8	12	16	0	4	8	12	16
<b>at 10°C</b>															
Control	4.31 <sup>a</sup> (±0.23)	3.87 <sup>a</sup> (±0.32)	3.66 <sup>a</sup> (±0.76)	3.00 <sup>b</sup> (±0.63)	1.31 <sup>c</sup> (±0.13)	4.22 <sup>a</sup> (±0.75)	3.33 <sup>b</sup> (±0.74)	3.24 <sup>b</sup> (±0.72)	2.73 <sup>c</sup> (±0.89)	1.51 <sup>d</sup> (±0.33)	4.32 <sup>a</sup> (±0.13)	3.67 <sup>b</sup> (±0.23)	3.63 <sup>b</sup> (±0.31)	3.16 <sup>b</sup> (±0.77)	1.31 <sup>c</sup> (±0.23)
5	4.41 <sup>a</sup> (±0.76)	3.95 <sup>abc</sup> (±0.41)	3.72 <sup>bc</sup> (±0.65)	3.66 <sup>c</sup> (±0.85)	2.11 <sup>d</sup> (±0.26)	4.17 <sup>a</sup> (±0.81)	3.83 <sup>b</sup> (±0.74)	3.48 <sup>b</sup> (±0.48)	2.83 <sup>c</sup> (±0.45)	2.31 <sup>c</sup> (±0.46)	4.45 <sup>a</sup> (±0.46)	4.13 <sup>a</sup> (±0.37)	4.00 <sup>a</sup> (±0.37)	3.66 <sup>b</sup> (±0.86)	2.11 <sup>c</sup> (±0.36)
10	4.53 <sup>a</sup> (±1.02)	4.05 <sup>a</sup> (±0.45)	3.86 <sup>b</sup> (±0.43)	3.60 <sup>b</sup> (±0.89)	2.23 <sup>c</sup> (±0.32)	4.32 <sup>a</sup> (±1.12)	3.91 <sup>b</sup> (±0.71)	3.68 <sup>b</sup> (±0.23)	3.00 <sup>c</sup> (±0.62)	2.23 <sup>d</sup> (±0.42)	4.23 <sup>a</sup> (±0.52)	4.00 <sup>a</sup> (±0.90)	3.83 <sup>b</sup> (±0.58)	3.76 <sup>b</sup> (±0.37)	2.23 <sup>c</sup> (±0.52)
15	4.37 <sup>a</sup> (±0.41)	4.00 <sup>ab</sup> (±0.36)	3.86 <sup>bc</sup> (±0.92)	3.56 <sup>c</sup> (±0.72)	2.67 <sup>d</sup> (±0.41)	3.97 <sup>a</sup> (±0.53)	3.86 <sup>a</sup> (±0.72)	3.83 <sup>a</sup> (±0.43)	3.16 <sup>b</sup> (±0.45)	2.17 <sup>c</sup> (±0.21)	4.37 <sup>a</sup> (±0.41)	3.99 <sup>ab</sup> (±0.50)	3.80 <sup>bc</sup> (±0.43)	3.73 <sup>c</sup> (±0.31)	3.07 <sup>d</sup> (±0.31)
20	4.17 <sup>a</sup> (±0.72)	3.97 <sup>a</sup> (±0.38)	3.96 <sup>a</sup> (±0.86)	3.49 <sup>b</sup> (±0.94)	2.61 <sup>c</sup> (±0.72)	4.00 <sup>a</sup> (±0.58)	3.85 <sup>a</sup> (±0.56)	3.80 <sup>a</sup> (±0.78)	3.16 <sup>b</sup> (±0.81)	2.00 <sup>c</sup> (±0.72)	4.25 <sup>a</sup> (±0.72)	3.97 <sup>ab</sup> (±0.30)	3.86 <sup>b</sup> (±0.55)	3.76 <sup>c</sup> (±0.39)	3.14 <sup>c</sup> (±0.71)
Treatments mean	4.37 <sup>a</sup> (±0.73)	3.99 <sup>a</sup> (±0.40)	3.85 <sup>ab</sup> (±0.72)	3.58 <sup>b</sup> (±0.85)	2.41 <sup>c</sup> (±0.43)	4.12 <sup>a</sup> (±0.76)	3.86 <sup>ab</sup> (±0.68)	3.70 <sup>bc</sup> (±0.48)	3.04 <sup>c</sup> (±0.58)	2.18 <sup>d</sup> (±0.45)	4.32 <sup>a</sup> (±0.53)	4.02 <sup>a</sup> (±0.52)	3.87 <sup>b</sup> (±0.48)	3.73 <sup>b</sup> (±0.48)	2.64 <sup>c</sup> (±0.48)
<b>at 25°C</b>															
Control	4.41 <sup>a</sup> (±0.53)	3.93 <sup>ab</sup> (±0.83)	3.83 <sup>b</sup> (±0.75)	2.67 <sup>c</sup> (±0.80)	2.31 <sup>d</sup> (±0.23)	4.37 <sup>a</sup> (±0.31)	3.33 <sup>b</sup> (±0.35)	2.80 <sup>c</sup> (±0.74)	2.66 <sup>c</sup> (±0.83)	1.41 <sup>d</sup> (±0.23)	4.22 <sup>a</sup> (±0.23)	3.90 <sup>b</sup> (±0.45)	2.80 <sup>c</sup> (±0.69)	2.16 <sup>d</sup> (±0.78)	1.21 <sup>c</sup> (±0.13)
5	4.21 <sup>a</sup> (±0.74)	4.00 <sup>a</sup> (±0.65)	3.91 <sup>a</sup> (±0.54)	3.77 <sup>b</sup> (±0.51)	2.57 <sup>c</sup> (±0.76)	4.18 <sup>a</sup> (±0.66)	3.80 <sup>b</sup> (±0.56)	3.53 <sup>c</sup> (±0.37)	3.23 <sup>d</sup> (±0.59)	2.34 <sup>c</sup> (±0.76)	4.41 <sup>a</sup> (±0.56)	4.23 <sup>a</sup> (±0.37)	3.98 <sup>b</sup> (±0.26)	3.17 <sup>c</sup> (±0.73)	2.22 <sup>d</sup> (±0.26)
10	4.23 <sup>a</sup> (±0.82)	3.98 <sup>b</sup> (±0.54)	3.88 <sup>bc</sup> (±0.29)	3.65 <sup>c</sup> (±0.56)	2.93 <sup>d</sup> (±1.02)	4.53 <sup>a</sup> (±1.00)	3.77 <sup>b</sup> (±0.34)	3.68 <sup>b</sup> (±0.19)	3.00 <sup>c</sup> (±0.14)	2.23 <sup>d</sup> (±1.02)	4.33 <sup>a</sup> (±0.42)	4.17 <sup>ab</sup> (±0.15)	3.96 <sup>b</sup> (±0.68)	3.33 <sup>b</sup> (±0.11)	2.23 <sup>c</sup> (±0.12)
15	4.30 <sup>a</sup> (±0.43)	3.89 <sup>b</sup> (±0.34)	3.85 <sup>b</sup> (±0.37)	3.66 <sup>bc</sup> (±0.58)	3.07 <sup>c</sup> (±0.41)	4.06 <sup>a</sup> (±0.52)	3.63 <sup>b</sup> (±0.74)	3.55 <sup>bc</sup> (±0.39)	3.13 <sup>c</sup> (±0.27)	2.67 <sup>d</sup> (±0.41)	4.17 <sup>a</sup> (±0.31)	3.99 <sup>ab</sup> (±0.53)	3.76 <sup>b</sup> (±0.66)	3.63 <sup>b</sup> (±0.34)	2.97 <sup>c</sup> (±0.42)
20	4.45 <sup>a</sup> (±0.62)	4.07 <sup>a</sup> (±0.91)	3.98 <sup>ab</sup> (±0.57)	3.70 <sup>bc</sup> (±0.31)	3.10 <sup>c</sup> (±0.72)	4.08 <sup>a</sup> (±0.98)	3.57 <sup>b</sup> (±0.28)	3.43 <sup>bc</sup> (±0.36)	3.16 <sup>c</sup> (±0.49)	2.70 <sup>d</sup> (±0.72)	4.00 <sup>a</sup> (±0.52)	3.90 <sup>bc</sup> (±0.30)	3.65 <sup>cd</sup> (±0.41)	3.43 <sup>d</sup> (±0.43)	2.36 <sup>c</sup> (±0.32)
Treatments mean	4.30 <sup>a</sup> (±0.65)	3.98 <sup>ab</sup> (±0.61)	3.91 <sup>bc</sup> (±0.44)	3.70 <sup>c</sup> (±0.49)	2.92 <sup>d</sup> (±0.73)	4.21 <sup>a</sup> (±0.79)	3.69 <sup>b</sup> (±0.48)	3.55 <sup>b</sup> (±0.33)	3.13 <sup>b</sup> (±0.37)	2.48 <sup>c</sup> (±0.73)	4.23 <sup>a</sup> (±0.45)	4.07 <sup>ab</sup> (±0.34)	3.84 <sup>b</sup> (±0.50)	3.39 <sup>b</sup> (±0.40)	2.45 <sup>c</sup> (±0.28)

Table 1. (Cont'd.)

Gum concentration (%)	Storage period (days)									
	Appearance					Overall acceptability				
	0	4	8	12	16	0	4	8	12	16
at 10°C										
Control	4.31 <sup>a</sup> (±0.23)	3.86 <sup>b</sup> (±0.41)	3.84 <sup>b</sup> (±0.41)	3.00 <sup>c</sup> (±0.63)	2.31 <sup>d</sup> (±0.33)	4.11 <sup>a</sup> (±0.53)	3.67 <sup>b</sup> (±0.34)	3.86 <sup>b</sup> (±0.65)	3.00 <sup>c</sup> (±0.87)	2.24 <sup>d</sup> (±0.23)
5	4.11 <sup>a</sup> (±0.76)	3.86 <sup>b</sup> (±0.38)	3.67 <sup>bc</sup> (±0.46)	3.33 <sup>c</sup> (±0.91)	2.91 <sup>d</sup> (±0.56)	4.17 <sup>a</sup> (±0.66)	3.87 <sup>b</sup> (±0.58)	3.66 <sup>bc</sup> (±0.49)	3.50 <sup>c</sup> (±0.99)	2.89 <sup>d</sup> (±0.76)
10	4.23 <sup>a</sup> (±1.02)	3.60 <sup>b</sup> (±0.83)	3.50 <sup>b</sup> (±0.63)	3.38 <sup>b</sup> (±0.78)	3.00 <sup>c</sup> (±0.42)	4.43 <sup>a</sup> (±0.52)	3.85 <sup>b</sup> (±0.47)	3.67 <sup>bc</sup> (±0.21)	3.50 <sup>cd</sup> (±0.37)	3.13 <sup>d</sup> (±0.32)
15	4.00 <sup>a</sup> (±0.41)	3.97 <sup>ab</sup> (±0.81)	3.83 <sup>b</sup> (±0.29)	3.53 <sup>c</sup> (±0.44)	3.07 <sup>d</sup> (±0.31)	3.99 <sup>a</sup> (±0.21)	3.86 <sup>a</sup> (±0.53)	3.83 <sup>ab</sup> (±0.67)	3.60 <sup>b</sup> (±0.79)	3.19 <sup>c</sup> (±0.61)
20	4.00 <sup>a</sup> (±0.72)	4.00 <sup>a</sup> (±0.52)	3.85 <sup>ab</sup> (±0.39)	3.33 <sup>b</sup> (±0.22)	3.10 <sup>b</sup> (±0.72)	4.05 <sup>a</sup> (±0.82)	4.00 <sup>a</sup> (±0.49)	3.86 <sup>b</sup> (±0.27)	3.67 <sup>b</sup> (±0.13)	3.34 <sup>c</sup> (±0.35)
Treatments mean	4.08 <sup>a</sup> (±0.73)	3.86 <sup>ab</sup> (±0.67)	3.71 <sup>b</sup> (±0.44)	3.39 <sup>bc</sup> (±0.59)	3.02 <sup>c</sup> (±0.50)	4.16 <sup>a</sup> (±0.55)	3.90 <sup>bc</sup> (±0.52)	3.76 <sup>c</sup> (±0.41)	3.57 <sup>cd</sup> (±0.57)	3.14 <sup>d</sup> (±0.51)
at 25°C										
Control	4.21 <sup>a</sup> (±0.13)	3.83 <sup>b</sup> (±0.07)	3.50 <sup>c</sup> (±0.36)	3.10 <sup>d</sup> (±0.09)	2.31 <sup>e</sup> (±0.23)	4.31 <sup>a</sup> (±0.73)	3.33 <sup>b</sup> (±0.98)	3.06 <sup>b</sup> (±0.68)	3.00 <sup>b</sup> (±0.82)	2.08 <sup>c</sup> (±0.13)
5	4.13 <sup>a</sup> (±0.76)	3.50 <sup>b</sup> (±0.19)	3.13 <sup>b</sup> (±0.27)	2.66 <sup>b</sup> (±0.65)	2.60 <sup>b</sup> (±0.76)	4.28 <sup>a</sup> (±0.46)	3.50 <sup>b</sup> (±0.44)	3.13 <sup>b</sup> (±0.83)	2.83 <sup>c</sup> (±0.58)	2.26 <sup>c</sup> (±0.36)
10	4.25 <sup>a</sup> (±0.62)	3.84 <sup>b</sup> (±0.34)	3.77 <sup>b</sup> (±0.24)	2.83 <sup>c</sup> (±0.39)	2.73 <sup>c</sup> (±1.02)	4.63 <sup>a</sup> (±0.92)	4.00 <sup>b</sup> (±0.66)	3.38 <sup>c</sup> (±0.47)	3.33 <sup>c</sup> (±0.69)	2.83 <sup>d</sup> (±0.12)
15	4.18 <sup>a</sup> (±0.41)	3.98 <sup>a</sup> (±0.37)	3.67 <sup>b</sup> (±0.37)	2.93 <sup>c</sup> (±0.47)	2.91 <sup>c</sup> (±0.41)	4.15 <sup>a</sup> (±0.81)	3.93 <sup>ab</sup> (±0.88)	3.87 <sup>b</sup> (±0.73)	3.00 <sup>c</sup> (±0.75)	2.99 <sup>c</sup> (±0.31)
20	4.00 <sup>a</sup> (±0.82)	3.90 <sup>a</sup> (±0.43)	3.13 <sup>b</sup> (±0.46)	2.91 <sup>c</sup> (±0.88)	2.88 <sup>c</sup> (±0.72)	4.10 <sup>a</sup> (±0.52)	3.83 <sup>b</sup> (±0.84)	3.00 <sup>c</sup> (±0.89)	2.83 <sup>d</sup> (±0.66)	2.50 <sup>e</sup> (±0.11)
Treatments mean	4.14 <sup>a</sup> (±0.65)	3.81 <sup>b</sup> (±0.33)	3.43 <sup>b</sup> (±0.34)	2.83 <sup>c</sup> (±0.60)	2.78 <sup>d</sup> (±0.73)	4.29 <sup>a</sup> (±0.68)	3.82 <sup>b</sup> (±0.71)	3.35 <sup>c</sup> (±0.73)	2.99 <sup>c</sup> (±0.67)	2.65 <sup>d</sup> (±0.23)

Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at  $p \leq 0.05$  as assessed by Duncan's multiple range test

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