Efficacy of pink guava pulp as an antioxidant in raw pork emulsion

Serlene Joseph · Manish K. Chatli · Ashim K. Biswas · Jhari Sahoo

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Abstract Lipid oxidation-induced quality problems can be minimized with the use of natural antioxidants. The antioxidant potential of pink guava pulp (PGP) was evaluated at different levels (0%; C, 5.0%; T-1, 7.5%; T-2 and 10.0%; T-3) in the raw pork emulsion during refrigerated storage of 9 days under aerobic packaging. Lycopene and β -carotene contents increased ($P<0.05$) with PGP levels. The redness (a^*) increased ($P<0.05$), whereas L^* decreased ($P<0.05$) with the incorporation of PGP. The visual colour and odour scores were greater ($P<0.05$) in PGP-treated products than control. Percent metmyoglobin formation was greater ($P<0.05$) in the control than PGP-treated products, and increased ($P<0.05$) during storage in all the treatments. Overall, peroxide value, thiobarbituric acid reactive substances and free fatty acid values were lower ($P<0.05$) in PGP-treated raw emulsion than control throughout storage period. Our results indicated that pink guava pulp can be utilized as antioxidants in raw pork products to minimize lipid oxidation, off-odour development, and surface discolouration.

Keywords Pink guava pulp · Lycopene · Colour · Natural antioxidant · Raw pork emulsion · β -carotene

Colour and flavour act as first stimuli for the consumer's acceptance of the meat and meat products. These sensory attributes are directed by lipid oxidation and indicate the storage life of meat and meat products. Surface colour and

odour of fresh meat is influenced by various factors including packaging methods, light exposure, time and temperature regime during storage and the presence of oxidants and antioxidants. Consequently, various artificial antioxidants have been added traditionally to preserve colour and odour profile of meat and meat products. These additives include sodium erythrobate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), propyl gallate, nitrite (NO_2) and nitrate (NO_3) (Valencia et al. 2007). The use of these compounds is debated due to carcinogenicity and growing consumer interest to natural ones (Fellenberg and Speisky 2006). This has fostered research on the screening of vegetable and fruit sources for identifying new antioxidants, such as extracts of rosemary (Han and Rhee 2005), grape seeds (Ahn et al. 2004), garlic (Sallam et al. 2004), green tea (Hassan and Fan 2005), cloud berry, beetroot and willow herb (Rey et al. 2005).

Red-fleshed guava (*Psidium guajava* Linn.) contains ascorbic acid and several carotenoids such as phytofluene, β -carotene, β -cryptoxanthin, γ -carotene, lycopene, rubixanthin, cryptoflavin, lutein, and neochrome (Mercadante et al. 1999). Among these carotenoids, lycopene is a potent quencher of singlet oxygen and scavenger of peroxy radicals (DiMasico et al. 1989). The antioxidant activity of lycopene is twice as of β -carotene and ten times greater than α -tocopherol (Shi and Maguer 2000). Phenolic compounds such as myricetin and apigenin (Miean and Mohamed 2001), ellagic acid and anthocyanins (Misra and Seshadri 1968) are also present at high levels in guava fruits. Guava contains an amazing amount of phytochemicals including tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, and lectins. The antioxidant property of guava is present in its peel and pulp due to vitamin C and carotenoids (Bashir and AbuGoukh

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2003). The leaves of guava are also rich in flavonoids, in particular quercetin, which has demonstrated antibacterial activity and is thought to contribute to the antidiarrheal effect of guava (Bashir and AbuGoukh 2003). Escrig et al. 2001 reported that the addition of peel and pulp fractions of guava can be used to obtain antioxidant dietary fiber (AODF), a new product which combines the properties of dietary fiber and antioxidant compounds. Therefore, guava can be used as a realistic approach to increase dietary antioxidant intake. However, the scientific information on the use of guava as an antioxidant in meat products is very limited. Hence, the present study was conducted to evaluate pink guava pulp as a natural antioxidant in raw pork emulsion during refrigerated storage under aerobic packaging.

Materials and methods

Materials and chemicals

The fresh ripened pink guavas (*Psidium guajava*) of uniform size, free from any visible defect were procured from farm at Gudalur, Tamil Nadu, India in the months of September and October. The skin and seeds were separated, and the pulp was packed in low density polyethylene (LDPE) bags and stored at -18°C until used.

Three castrated male pigs, Large White Yorkshire, (Age 8–10 months, weighing 90–100 kg) were procured from Instructional Livestock farm of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The animals were humanely slaughtered in the experimental abattoir following animal welfare and ethics protocols approved by GADVASU, Animal Ethical Committee. The dressed carcasses were brought to the laboratory immediately and chilled at $4\pm 1^{\circ}\text{C}$ for 18 h and then deboned manually. The deboned meat was packaged in LDPE bags and stored in a deep freezer at -18°C , until further used.

The chemicals of analytical grades were purchased from standard firms, whereas lycopene and thiobarbituric acid standards were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Preparation of meat emulsion

Frozen deboned lean pork meat free from connective tissue and fat was cut into small cubes of size $2.54\text{ cm}\times 2.54\text{ cm}\times 2.54\text{ cm}$ and minced to a particle size of 6 mm through a stainless steel meat mincer (KL-32, Kalsi, Ludhiana, India). Back fat trimmings were minced separately to the same particle size. Pink guava pulp (PGP) free from peel and seeds was grinded in a domestic grinder (Inalsa Technologies, New Delhi, India) to prepare a paste. Four different treatments; C, T-1, T-2 and T-3 of pork meat emulsion were prepared. All the batches contained 78% lean meat, 10% back fat and 2% NaCl. Control (C) was prepared using 0% PGP+10% water, T-1 (5% PGP+5% water), T-2 (7.5% PGP+2.5% water) and T-3 (10% PGP+0% water). All the ingredients were mixed manually for 5 min and subsequently in a domestic kneader-cum-blender (Inalsa Technologies, New Delhi, India) for 60 s to prepare emulsion. The different groups were packaged in LDPE bags, thermally sealed and stored for 9 days in a refrigerator. The samples were drawn on day 1, 3, 5, 7, and 9 and analyzed for different physico-chemical and colour parameters.

Physico-chemical attributes measurements

Meat pH and titratable acidity

Ten gram of emulsion was homogenized with 50 ml distilled water, and the pH of the suspension was recorded using digital pH meter (SAB 5000, LABINDIA, New Delhi, India).

The titratable acidity was measured as described by Shelef and Jay (1970). Ten gram of meat emulsion was blended with 200 ml distilled water, and the volume was made up to 250 ml. The slurry was filtered through Whatman filter paper No. 2. Twenty-five milliliters of filtrate were collected, and 75 ml distilled water and three drops of 1% phenolphthalein indicator were added. The resulting solution was titrated against 0.1 N NaOH till the endpoint (pink colour) was achieved. The titratable acidity was calculated as given below and expressed as percent lactic acid.

$$\text{Titratable acidity} = (\text{ml of } 0.1\text{N NaOH} \times \text{meq wt. of lactic acid} \times 100) / \text{Weight of sample in g}$$

Active components measurements

β -carotene β -carotene content in meat emulsion was determined following AOAC method (1995) with suitable

modifications. A representative sample (1 g) of the meat emulsion was triturated with 20 ml of acetone in the presence of anhydrous sodium sulfate. The sample was quantitatively transferred in a polypropylene centrifuge tube and held at $4\pm$

1 °C for 15 min with occasional shaking. Then, it was centrifuged at 3200 g for 10 min (MP400R, Eltek, India Limited, Delhi, India) at 4 °C and the supernatant was decanted in a separate tubes and the samples were re-extracted again with 20 ml of acetone. Both the supernatants were combined and then passed through the Whatman's filter paper No.42. The absorbance of the extract was determined at 449 nm wavelength in a UV–VIS spectrophotometer (SL-159, Elico India Limited, Hyderabad, India). The concentration of β -carotene was determined by an external standard method substituting respective absorbance in the linear regression formula ($y = 0.087x$ and $r^2 = 0.9747$; where y =absorbance, x = β -carotene concentration, and r^2 =correlation coefficient)

Lycopene The lycopene content was measured using the spectrophotometric method described by Fish et al. (2002) with minor modifications. A meat sample of 2 g was taken in amber coloured screw top vials and 5 ml of 0.05% (w/v) BHT in acetone, 5 ml of 95% ethanol and 10 ml of hexane were added. The vials were placed in ice, and stirred on a magnetic stirring plate for 45 min. Then, 3 ml of deionized water were added to each vial, and again the samples were shaken for about 10 min, while keeping still in ice for few minutes to allow the separation of both the phases. The absorbance of the hexane (upper layer) was recorded at 503 nm versus a blank of hexane solvent using a UV–VIS Spectrophotometer (SL-159, Elico India Limited, Hyderabad, India). The lycopene content of the tissue was then estimated by the following formula: where, A_{503} is the absorbance at 503 nm and 31.2 is the constant

$$\text{Lycopene (mg/kg sample)} = (A_{503} \times 31.2) / \text{Weight of sample in kg}$$

Instrumental colour profile

At the scheduled interval during storage, emulsions were removed from the packages, and the samples were spread in Petri dishes of 10 cm diameter and 1.2 cm thickness. CIE L^* (lightness), a^* (redness), and b^* (yellowness) values, and hue angle were measured at three random locations on each Petri dish using a HunterLab MiniScan XE Plus colourimeter (HunterLab Associates, Reston, VA, USA) with illuminant D65, 2.54-cm diameter aperture, and 2° standard observer (AMSA 1991).

Storage stability indicators

Percent metmyoglobin formation The percent metmyoglobin of pork emulsion was measured using the method of

Trout (1989). The optical density was measured in a UV–VIS spectrophotometer (SL-159, Elico India Limited, Hyderabad, India) at 525, 572 and 700 nm. Metmyoglobin percent was calculated using the formula of Krzywicki (1979)

$$\text{Percent metmyoglobin} = 100 \times \{1.395 - [(A_{572} - A_{700}) / (A_{525} - A_{700})]\}$$

Thiobarbituric acid reactive substances (TBARS) value TBARS was measured using extraction method described by Witte et al. 1970. Absorbance (O.D.) was measured at a fixed wavelength of 532 nm using UV–VIS spectrophotometer (SL-159, Elico India Limited, Hyderabad, India). TBARS value was calculated as mg malonaldehyde per kg of sample.

Peroxide value (PV) measurements The peroxide value was measured as per procedure described by Koniecko (1979) with suitable modifications. The peroxide value (meq/kg of the meat) was calculated as per the following formula

$$\text{PV (meq/kg sample)} = 1000 \times (\text{ml of 0.1N sodium thiosulfate} / \text{Weight of sample in g})$$

Free fatty acids (FFA) Free fatty acids were estimated using method as described by Koniecko (1979) and expressed in percentage and calculated as

$$\text{FFA (\%)} = 100 \times (\text{ml of 0.1N alcoholic KOH} \times 0.282) / \text{Weight of sample in g}$$

Extract release volume (ERV) The standard method of Jay (1964) was followed. Fifteen gram of meat emulsion was blended with 60 ml of 0.05 M PBS (pH 5.8) for 2 min in a tissue homogenizer. The homogenate was filtered through Whatman filter paper No.1. Filtration was carried out for 15 min and filtrate was collected in a measuring cylinder. The volume of the extract was measure of ERV and expressed as ml.

Sensory colour and odour measurements

The visual colour and odour scores were determined by using the 5-point descriptive score card. The colour and odour assessment panel consisted of seven untrained members selected among the students and faculty members of the university. They were briefed on the visual colour and odour of the pork meat samples, and provided with a fresh pork meat as a reference sample at different stages of storage. They

were also detailed on the color and odour score card. The panel members were presented test meat samples after assigning suitable codes. The samples were evaluated for various attributes of colour (where 1 = grayish pink, 2 = light reddish pink, 3 = moderately pink, 4 = dark reddish pink and 5 = purplish red) and odour score card (where 1 = very unpleasant, 2 = moderately unpleasant, 3 = moderately pleasant, 4 = pleasant and 5 = very pleasant).

Statistical analysis

Data were analyzed statistically on SPSS-16.0 software packages (SPSS Inc. Chicago, IL, USA) as per standard methods. Duplicate samples were drawn for each parameter, and the experiments were repeated three times to provide six replicates ($n=6$). The instrumental colour evaluation was carried out with four samples and was repeated three times, leading to total 12 observations ($n=12$). Visual colour and odour evaluation was performed by a seven-member panel in each trial leading to 21 observations ($n=21$). Means between the periods of storage, between treatments and within treatments were compared by two-way analysis of variance and critical difference test. The statistical significance was estimated at 5% level ($P<0.05$) and evaluated with Duncan's Multiple Range Test.

Results and discussion

Meat pH and Titratable acidity

The pH of raw pork emulsion varied ($P<0.05$) with the level of incorporation of PGP and storage period (Table 1). The pH was lower ($P<0.05$) in pink guava-treated pork emulsion than control and this was the lowest ($P<0.05$) in T-3. This might be due to the pH of PGP (4.96–5.0), and level of incorporation in pork emulsion. The emulsion pH followed a declining trend up to day 7, and thereafter increased ($P<0.05$) on day 9 of storage in T-2 and T-3. However, the pH decreased ($P<0.05$) up to day 5 and thereafter increased ($P<0.05$) during storage in case of T-1. The pH followed an increasing trend throughout storage in control emulsion. The changes in pH during storage might be due to various factors including pH of the PGP, acidity produced by the bacterial action on the fruit sugars and muscle glucose, and accumulation of the metabolites due to bacterial spoilage in pork emulsion (de Pablo et al. 1989; Kumar and Sharma 2004).

The changes in pH were directed by the titratable acidity of the emulsion. The titratable acidity (Table 1) was greater ($P<0.05$) for T-3 than T-1 and T-2. The titratable acidity increased ($P<0.05$) during storage and corresponded with the decrease in pH. Furthermore, control samples demonstrated lower ($P<0.05$) titratable acidity than the guava-treated emulsions throughout the storage, due to higher pH.

Beta-carotene and lycopene contents

Beta-carotene and lycopene contents were not detected in control emulsion. Results (Table 2) revealed that β -carotene and lycopene contents are concentration dependent and increased ($P<0.05$) with the increase in level of incorporation of PGP in the emulsion. β -carotene followed a declining trend during storage period. The decrease was estimated as 25%, 32.14% and 26.31% for T-1, T-2 and T-3, respectively from day 1 to day 9 of storage period. The decrease might be due to chemical breakdown of β -carotene (Negi and Roy 2003) occurring with lipid oxidation process in pork emulsion. Lycopene content increased almost 131.42% from T-1 to T-3 with the increase in the level of incorporation of PGP from 5% to 10%. The lycopene content also followed similar declining trend as per β -carotene during storage and the percent decrease was estimated as 19%, 30.05% and 21.76% for T-1, T-2 and T-3, respectively. Similar observations were recorded by Osterlie and Lerfall (2005) in meat farce containing lycopene. The rate of decrease of lycopene corresponds to oxidation of the carotenoid pigments during storage (Calvo et al. 2008).

Colour quality parameters

CIE L^* , a^* , b^* and hue angle values of control and guava-treated pork emulsion are presented in Table 3. L^* value was greater ($P<0.05$) in control than all the treatments. These results indicated that the emulsions containing guava products were darker than the controls. L^* value increased ($P<0.05$) on day 3 of storage and thereafter, declined on further storage in all the treatments. The initial increase in L^* value might be due to the uniform distribution and breakdown of tissue matrix of the PGP. Candogan (2002) and Calvo et al. (2008) also reported a decrease in L^* value with the incorporation of lycopene rich tomato peel powder in beef and beef products.

Surface redness is the most important characteristic influencing the purchase decisions because consumers often consider this as an indicator of wholesomeness. Surface redness (a^* value) was greater ($P<0.05$) in pink guava-treated raw pork emulsion than control throughout the storage. The surface redness of pork emulsion increased with the increase in the level of incorporation of PGP. The redness was lower ($P<0.05$) in T-1 than T-3 throughout storage except on day 1. This can be correlated with the lycopene content in the pork emulsion, which is a potent colourant (Osterlie and Lerfall 2005). Similar to L^* , a^* value also increased on day 3, and thereafter, decreased ($P<0.05$) throughout storage in all other treatments. Candogan (2002) reported an increase in a^* value with the incorporation of tomato paste in beef patties. However, the literature does not address on the effect of PGP on instrumental colour profile of meat system.

Table 1 Changes in pH and titratable acidity of raw pork emulsion incorporated with different levels of pink guava pulp during refrigerated storage under aerobic packaging

Treatments	Day 1	Day 3	Day 5	Day 7	Day 9
pH ¹					
C	5.7±0.01 ^{ya}	5.8±0.02 ^{yb}	5.9±0.02 ^{yc}	6.0±0.02 ^{yd}	6.1±0.02 ^{ye}
T-1	5.6±0.01 ^{xa}	5.6±0.01 ^{xa}	5.6±0.01 ^{xa}	5.7±0.03 ^{xb}	5.7±0.02 ^{xb}
T-2	5.6±0.01 ^{xb}	5.6±0.02 ^{wxb}	5.6±0.02 ^{xb}	5.5±0.02 ^{wa}	5.5±0.01 ^{wa}
T-3	5.5±0.02 ^{wa}	5.5±0.02 ^{wa}	5.5±0.01 ^{wa}	5.5±0.02 ^{wa}	5.5±0.02 ^{wa}
Titratable acidity ¹					
C	0.054±0.001 ^{wxc}	0.053±0.001 ^{wc}	0.049±0.001 ^{wb}	0.042±0.003 ^{wa}	0.038±0.002 ^{wa}
T-1	0.051±0.002 ^{wa}	0.060±0.002 ^{xb}	0.063±0.002 ^{xb}	0.057±0.002 ^{xb}	0.055±0.004 ^{xab}
T-2	0.053±0.002 ^{wa}	0.058±0.002 ^{xab}	0.062±0.003 ^{xb}	0.065±0.003 ^{yb}	0.060±0.002 ^{yab}
T-3	0.057±0.003 ^{xa}	0.061±0.003 ^{xa}	0.065±0.002 ^{xab}	0.069±0.003 ^{yb}	0.061±0.004 ^{ya}

¹ n=6; C=Control, T-1=5% pink guava pulp; T-2=7.5% pink guava pulp; T-3=10% pink guava pulp

^{a-c} Mean±Standard Error for the same trait in a row without a common letter differ ($P<0.05$)

^{w-y} Mean±Standard Error for the same trait in a column without a common letter differ ($P<0.05$)

There was no significant ($P<0.05$) effect on b^* value (yellowness) of raw pork emulsion with the change in the level of PGP. It was greater ($P<0.05$) in control than PGP-treated products on day 9 of storage. The b^* value increased on day 3 and thereafter decreased ($P<0.05$) during storage in all the treatments. On the contrary, García et al. (2009) and Selgas et al. (2009) reported increase in b^* values with the increase in lycopene rich dried tomato powder in hamburgers. However, they also reported decrease in b^* values during storage period. The variation in the results might be due to lower level of lycopene in pink guava pulp than tomato peel powder.

Hue angle, which correlates with visual assessment of meat discolouration (Giroux et al. 2001), was greater ($P<0.05$) in control than in the antioxidant treatments on day 7 and day 9 of storage (Table 3). This could be attributed to the greater a^* values in antioxidant treatments, which confirmed the colour-improving effect of pink guava pulp in pork emulsion. Furthermore, this observation concurred with the results of visual colour evaluation. Among the antioxidant treatments, hue angle was greater ($P<0.05$) in T-2 than in T-1 and T-3. Hue angle did not varied ($P<0.05$) during the entire storage period for pink guava-treated products.

Table 2 Changes in β -carotene, lycopene content and percent metmyoglobin of raw pork emulsion incorporated with different levels of pink guava pulp during refrigerated storage under aerobic packaging

Treatments	Day 1	Day 3	Day 5	Day 7	Day 9
β -carotene (mg/kg) ¹					
T-1	0.16±0.01 ^{wab}	0.18±0.03 ^{wb}	0.13±0.01 ^{wa}	0.12±0.01 ^{wa}	0.12±0.01 ^{wa}
T-2	0.28±0.01 ^{xb}	0.26±0.02 ^{xb}	0.27±0.02 ^{xb}	0.20±0.02 ^{xa}	0.19±0.01 ^{xa}
T-3	0.38±0.00 ^{yb}	0.39±0.03 ^{yb}	0.34±0.01 ^{yab}	0.32±0.03 ^{yab}	0.28±0.02 ^{ya}
Lycopene (mg/kg) ¹					
T-1	1.9±0.09 ^{wab}	2.1±0.13 ^{wb}	1.8±0.14 ^{wa}	1.7±0.18 ^{wa}	1.5±0.16 ^{wa}
T-2	3.4±0.21 ^{xb}	3.3±0.22 ^{xb}	2.3±0.12 ^{xab}	2.5±0.18 ^{xab}	2.4±0.21 ^{xa}
T-3	4.4±0.23 ^{yb}	4.4±0.30 ^{yb}	3.8±0.16 ^{yab}	3.7±0.23 ^{ya}	3.5±0.12 ^{ya}
Percent metmyoglobin					
C	57.9±2.19 ^{xa}	65.9±2.15 ^{xb}	72.2±0.51 ^{yc}	80.5±1.39 ^{yd}	86.2±1.27 ^{ze}
T-1	53.8±1.19 ^{wxa}	57.9±2.31 ^{wxa}	62.5±0.94 ^{xb}	72.1±1.58 ^{xc}	79.1±1.56 ^{yd}
T-2	49.1±2.51 ^{wa}	53.8±1.22 ^{wa}	60.3±1.04 ^{xb}	69.8±2.64 ^{xc}	74.9±1.21 ^{xc}
T-3	47.6±1.33 ^{wa}	51.4±0.92 ^{wab}	54.1±0.71 ^{wb}	60.7±2.23 ^{wc}	63.5±1.12 ^{wc}

β -carotene and Lycopene were not detected in control (C) sample

¹ n=6; ND=Not Detected; C=Control, T-1=5% pink guava pulp; T-2=7.5% pink guava pulp; T-3=10% pink guava pulp

^{a-e} Mean±Standard Error for the same trait in a row without a common letter differ ($P<0.05$)

^{w-z} Mean±Standard Error for the same trait in a column without a common letter differ ($P<0.05$)

Table 3 Effect of different levels of pink guava pulp on the colour quality parameters of raw pork emulsion during refrigerated storage under aerobic packaging

Treatments	Day 1	Day 3	Day 5	Day 7	Day 9
<i>L</i> * ¹					
C	51.9±0.70 ^{yb}	54.0±0.43 ^{yc}	53.7±0.48 ^{yc}	52.1±0.40 ^{xb}	50.0±0.52 ^{xa}
T-1	49.3±0.37 ^{xa}	51.5±0.33 ^{xb}	51.3±0.13 ^{xb}	49.4±0.46 ^{wa}	49.1±0.41 ^{wxa}
T-2	48.5±0.53 ^{xa}	51.5±0.39 ^{xb}	52.0±0.36 ^{xb}	50.7±0.47 ^{wxb}	48.1±0.40 ^{wa}
T-3	46.4±0.36 ^{wa}	51.8±0.26 ^{wb}	51.1±0.25 ^{wab}	49.9±0.32 ^{wc}	49.3±0.34 ^{wxc}
<i>a</i> * ¹					
C	4.1±0.10 ^{wc}	4.0±0.13 ^{wc}	2.9±0.14 ^{wb}	2.6±0.15 ^{wb}	1.8±0.10 ^{wa}
T-1	4.5±0.16 ^{wxc}	5.1±0.12 ^{xd}	4.0±0.10 ^{xb}	3.7±0.06 ^{xb}	2.6±0.12 ^{xa}
T-2	5.0±0.13 ^{xd}	5.5±0.10 ^{xyd}	4.2±0.10 ^{xb}	4.7±0.07 ^{yc}	3.3±0.09 ^{ya}
T-3	5.1±0.12 ^{xbc}	5.6±0.14 ^{yc}	5.0±0.17 ^{yab}	4.5±0.11 ^{yb}	3.9±0.20 ^{ya}
<i>b</i> * ¹					
C	9.4±0.23 ^{wb}	9.5±0.14 ^{xb}	9.5±0.12 ^{xb}	9.1±0.14 ^{xab}	8.7±0.15 ^{xa}
T-1	9.1±0.17 ^{wc}	9.4±0.10 ^{wxd}	8.8±0.12 ^{wb}	8.6±0.09 ^{wab}	8.4±0.11 ^{wa}
T-2	9.1±0.37 ^{wbc}	9.2±0.10 ^{wc}	8.8±0.17 ^{wb}	8.9±0.20 ^{wxb}	8.3±0.07 ^{wa}
T-3	9.0±0.10 ^{wbc}	9.2±0.16 ^{wc}	9.1±0.19 ^{wxbc}	8.8±0.10 ^{wxb}	8.3±0.12 ^{wa}
Hue angle ¹					
C	67.2±0.44 ^{wxa}	68.7±0.61 ^{xa}	69.0±0.86 ^{yab}	69.8±0.49 ^{yb}	68.5±0.53 ^{xa}
T-1	64.4±1.03 ^{wa}	62.1±1.29 ^{wa}	61.1±0.67 ^{wa}	62.6±0.51 ^{wa}	62.5±0.85 ^{wa}
T-2	69.6±0.30 ^{xa}	68.4±0.69 ^{xa}	68.0±0.55 ^{ya}	67.8±0.41 ^{xa}	68.2±0.61 ^{xa}
T-3	65.5±0.85 ^{wa}	63.9±0.60 ^{wa}	65.5±0.91 ^{xa}	65.3±0.77 ^{xa}	67.4±1.39 ^{wa}

¹ n=12; C=Control, T-1=5% pink guava pulp; T-2=7.5% pink guava pulp; T-3=10% pink guava pulp^{a-c} Mean±Standard Error for the same trait in a row without a common letter differ (*P*<0.05)^{w-y} Mean±Standard Error for the same trait in a column without a common letter differ (*P*<0.05)

These results are in consonance with the measurements for *a** and *b** value.

Storage stability indicators

Percent metmyoglobin formation The formation of brown metmyoglobin is the indicator of discolouration of meat. The percent metmyoglobin formation was greater (*P*<0.05) in control than pink guava-treated raw pork emulsion (Table 2). This was indirectly proportional to the level of PGP in pork emulsion and the lowest metmyoglobin percentage was recorded for T-3 and highest for T-1. The percent metmyoglobin formation increased (*P*<0.05) during storage period in all the treatments. The observed lower metmyoglobin formation in the antioxidant-treated samples than controls could be attributed to the decrease in lipid oxidation, which is a major factor promoting myoglobin oxidation (Faustman et al. 2010). Escrig et al. (2001) also detailed the antioxidant effect of guava fruit due to the presence of extractable polyphenols (2.262–7.79%).

Peroxide value The PV was lower (*P*<0.05) in T-2 and T-3 than control throughout the storage, whereas T-1 exhibited PV comparable to control during entire storage period except on

day 9 (Table 4). Nonetheless, PV in pink guava-treated emulsions was numerically lower than in control. PV increased (*P*<0.05) during the storage in all the treatments. The greater PV of T-1 may be explained on the basis of its greater pH and lower level of active components than T-2 and T-3. Osterlie and Lerfall (2005) also argued that low pH could minimize hydrolytic activity of microorganisms and reduce oxidative rancidity in meat products.

TBARS Perusal of Table 4 revealed that TBARS was greater (*P*<0.05) in control than all the pink guava-treatments except T-1. However, TBARS were greater (*P*<0.05) for control than all pink guava-treated raw pork emulsion on day 9 of storage. TBARS increased (*P*<0.05) during storage irrespective of the treatments. In control, TBARS was greater than the threshold limit of 2 mg MDA/kg (Eyler, and Oztan 2011; Greene, and Cumuze 1982) on day 9 of storage, whereas it was lower than the threshold limit in all antioxidant-treated products throughout the storage. These results indicated the oxidative stability derived from PGP consisting of lycopene, β-carotene and other polyphenols including ascorbic acid (200–300 mg/100 g) (Padula and Rodryuez Amaya 1986). TBARS values were lower (*P*<0.05) in T-3 than T-1 on the first and last days of the

Table 4 Effect of different levels of pink guava pulp on the oxidative stability of raw pork emulsion during refrigerated storage under aerobic packaging

Treatments	Day 1	Day 3	Day 5	Day 7	Day 9
Peroxide value (meq/kg) ¹					
C	5.7±0.12 ^{xa}	6.0±0.35 ^{xa}	7.6±0.14 ^{yb}	7.8±0.12 ^{yb}	10.6±0.28 ^{yc}
T-1	5.5±0.16 ^{xa}	5.7±0.29 ^{wxa}	7.2±0.12 ^{yb}	7.4±0.19 ^{xyb}	9.8±0.31 ^{xc}
T-2	5.2±0.13 ^{wa}	5.3±0.42 ^{wa}	6.6±0.16 ^{xb}	7.2±0.16 ^{xc}	8.5±0.32 ^{wxd}
T-3	4.6±0.11 ^{wa}	4.8±0.36 ^{wa}	5.1±0.19 ^{wb}	6.3±0.16 ^{wc}	7.8±0.24 ^{wd}
Free fatty acids (%) ¹					
C	0.085±0.004 ^{ya}	0.097±0.007 ^{yb}	0.120±0.005 ^{yc}	0.131±0.004 ^{xd}	0.134±0.004 ^{xd}
T-1	0.077±0.002 ^{xa}	0.086±0.005 ^{xb}	0.095±0.004 ^{xb}	0.107±0.005 ^{wbc}	0.113±0.004 ^{wc}
T-2	0.076±0.003 ^{xa}	0.085±0.002 ^{xb}	0.095±0.003 ^{xc}	0.101±0.002 ^{wc}	0.108±0.005 ^{wd}
T-3	0.071±0.002 ^{wa}	0.068±0.004 ^{wb}	0.081±0.004 ^{wc}	0.105±0.003 ^{wcd}	0.110±0.007 ^{wd}
Extract release volume (ml) ¹					
C	49.3±0.33 ^{wc}	47.5±0.88 ^{wd}	44.5±0.76 ^{wc}	40.0±0.45 ^{wb}	36.7±0.80 ^{wa}
T-1	49.8±0.95 ^{wd}	47.3±0.67 ^{wcd}	46.3±0.21 ^{wxc}	44.8±0.52 ^{wxb}	40.8±0.60 ^{xa}
T-2	50.2±0.48 ^{wd}	48.5±0.76 ^{wc}	47.2±1.14 ^{xb}	45.2±0.83 ^{xb}	40.3±0.48 ^{xa}
T-3	49.0±0.36 ^{wd}	48.0±0.73 ^{wd}	44.8±1.25 ^{wc}	42.5±1.48 ^{wb}	39.3±0.84 ^{xa}
Thiobarbituric acid reactive substances value (mg malonaldehyde/kg) ¹					
C	0.82±0.09 ^{xa}	1.2±0.10 ^{xb}	1.5±0.09 ^{yc}	1.9±0.12 ^{yd}	2.3±0.11 ^{ze}
T-1	0.77±0.08 ^{xa}	0.91±0.10 ^{wxb}	1.3±0.05 ^{xy}	1.6±0.07 ^{xyd}	1.9±0.09 ^{ye}
T-2	0.62±0.05 ^{wa}	0.83±0.76 ^{wb}	1.2±0.09 ^{xc}	1.5±0.10 ^{xd}	1.7±0.07 ^{xe}
T-3	0.56±0.06 ^{wa}	0.81±0.04 ^{wb}	1.0±0.09 ^{wc}	1.2±0.05 ^{wd}	1.5±0.07 ^{we}

¹ n=6; C=Control, T-1=5% pink guava pulp; T-2=7.5% pink guava pulp; T-3=10% pink guava pulp^{a-e} Mean±Standard Error for the same trait in a row without a common letter differ (*P*<0.05)^{w-z} Mean±Standard Error for the same trait in a column without a common letter differ (*P*<0.05)

storage. These results have confirmed that the antioxidant effect of PGP is concentration dependent showing higher inhibition at 10% level of incorporation in raw pork emulsion.

Free fatty acids Similar to PV and TBARS, FFA content (Table 4) was greater (*P*<0.05) in control than all the pink guava-treated products. FFA increased (*P*<0.05) throughout storage period for all the treatments. Among treatments FFA

Table 5 Effect of different levels of pink guava pulp on the sensory quality of raw pork emulsion during refrigerated storage under aerobic packaging

Treatments	Day 1	Day 3	Day 5	Day 7	Day 9
Visual colour scores ¹					
C	2.5±0.18 ^{wb}	2.4±0.15 ^{wb}	2.3±0.16 ^{wb}	2.2±0.21 ^{wb}	1.7±0.10 ^{wa}
T-1	2.7±0.10 ^{wbc}	2.9±0.08 ^{xc}	2.8±0.11 ^{xb}	2.5±0.12 ^{wab}	2.3±0.10 ^{xa}
T-2	2.8±0.11 ^{wab}	3.0±0.00 ^{xb}	2.8±0.11 ^{xab}	2.5±0.18 ^{wa}	2.4±0.15 ^{xa}
T-3	3.4±0.10 ^{xb}	3.6±0.15 ^{yc}	3.3±0.10 ^{ybc}	3.0±0.00 ^{xb}	2.7±0.10 ^{xa}
Odour scores ¹					
C	4.0±0.00 ^{wd}	3.9±0.08 ^{wd}	3.5±0.00 ^{wc}	3.2±0.08 ^{wb}	2.7±0.10 ^{wa}
T-1	4.0±0.00 ^{wc}	3.9±0.08 ^{wc}	3.5±0.18 ^{wb}	3.3±0.11 ^{wab}	3.0±0.07 ^{xa}
T-2	4.0±0.00 ^{wd}	4.0±0.00 ^{wd}	3.6±0.08 ^{wc}	3.3±0.11 ^{wb}	3.0±0.04 ^{xa}
T-3	4.0±0.00 ^{wd}	3.9±0.08 ^{wd}	3.7±0.10 ^{wc}	3.3±0.10 ^{wb}	3.0±0.04 ^{xa}

¹ n=21; C=Control, T-1=5% pink guava pulp; T-2=7.5% pink guava pulp; T-3=10% pink guava pulp^{a-d} Mean±Standard Error for the same trait in a row without a common letter differ (*P*<0.05)^{w-y} Mean±Standard Error for the same trait in a column without a common letter differ (*P*<0.05)

was the lowest ($P<0.05$) in T-3 up to day 5 of storage. However, there was no significant ($P<0.05$) difference in FFA among treatments on the last day of storage. FFA was greater ($P<0.05$) in control than PGP-treatments throughout storage. This may be due to possible low lipolysis and lipolytic enzyme activity in pink guava-treated products, leading to low production of free fatty acids (Aguirrezabal et al. 2000).

Extract release volume The critical appraisal of the ERV results (Table 4) revealed that there was no significant ($P<0.05$) difference between control and PGP-treated products, as well as among treatments throughout storage. However, ERV followed a declining trend in all the treatments as well as control during storage period. This might be due to variation in composition, added moisture and changes occurred in protein structure and composition due to bacterial action during refrigerated storage under aerobic packaging (Shelef and Jay 1970).

Sensory colour and odour measurements

Visual colour scores Visual colour scores (Table 5) were significantly ($P<0.05$) greater in pink guava-treated products than control on day 3, 5 and 9 of storage. Among antioxidant-treatments visual colour scores were greater ($P<0.05$) in T-3 than T-1 and T-2. These results revealed that the effect of pink guava pulp on the colour of meat is concentration dependent. The colour scores followed a declining trend during storage, whereas the scores were greater ($P<0.05$) in PGP-treated product than control throughout storage. The sensory panelists rated all the treatments comparable on day 9 of storage. These results are in consonance with the findings of instrumental colour profile. Selgas et al. (2009) also reported increase in visual colour scores with the incorporation of lycopene in raw hamburgers.

Odour scores The sensory panelists recorded comparable ($P<0.05$) odour scores for all the antioxidant-treated pork emulsion as well as control. This indicated that the addition of PGP upto 10% level in pork emulsion did not have significant effect on the odour of pork emulsion. Mean odour scores followed a declining trend during storage. The odour scores were significantly ($P<0.05$) greater in PGP-treated products than control on day 9 of storage. However, sensory panelists did not record any off odour in treatments even on day 9 of storage. The observed odour scores were in agreement with the indicators for lipid oxidation (TBARS, PV, and FFA) in raw pork emulsion.

Conclusions

Pink guava pulp is the rich source of natural antioxidants such as β -carotene and lycopene, which can prevent lipid oxidation in complex food system. The results in the present study demonstrated that the addition of pink guava pulp improves colour and minimizes lipid oxidation in raw pork emulsion during refrigerated storage under aerobic packaging. Therefore, meat industry can effectively utilize 10% level of pink guava pulp to minimize oxidation-induced deteriorative changes in raw pork emulsion without compromising the sensory quality attributes.

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