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Anti-oxidant effect of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties

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ABSTRACT

To overcome the disadvantages of using synthetic anti-oxidants in meat products, an investigation was carried out to evaluate the anti-oxidant effect of extracts of fruit by-products viz., kinnow rind powder (KRP), pomegranate rind powder (PRP) and pomegranate seed powder (PSP) in goat meat patties. Total phenolics content, DPPH radical scavenging activity and effect of these extracts on instrumental color, sensory attributes and TBARS values during storage ($4 \pm 1^\circ\text{C}$) of goat meat patties were evaluated. Results showed that these extracts are rich sources of phenolic compounds having free radical scavenging activity. Hunter Lab *L* value significantly ($P < 0.05$) lowered in PRP followed by PSP and KRP patties. Sensory evaluation indicated no significant differences among patties. Further, a significant ($P < 0.5$) reduction in TBARS values (lipid oxidation) during storage of goat meat patties was observed in PRP, PSP and KRP as compared to control patties. Average TBARS values (mg/kg meat) during refrigerated storage ($4 \pm 1^\circ\text{C}$) were significantly lower in PRP, followed by PSP and KRP as compared to control. The overall anti-oxidant effect was in the order of PRP > PSP > KRP. It was concluded that extracts of above fruits by-product powders have potential to be used as natural anti-oxidants in meat products.

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1. Introduction

Lipid oxidation and auto-oxidation are major causes of deterioration and reduced shelf life of meat products. Lipid oxidation may produce changes in meat quality parameters such as color, flavour, odor, texture and even nutritional value (Fernandez, Perez-Alvarez, & Fernandez-Lopez, 1997). Minced meat and meat products undergo oxidative changes more quickly as grinding exposes lipid membranes to metal oxidation catalysts. Synthetic anti-oxidants like BHT and BHA have been successfully used to prevent the oxidation in meat products. However, the synthetic anti-oxidants used currently have been found to exhibit various health effects (Shahidi, Janita, & Wanasundara, 1992). Reports of adverse health effects of these synthetic chemicals have led to growing interest in natural sources of anti-oxidants in meat products. In addition there has been growing interests in natural anti-oxidants because of their safety, consumer acceptability and greater application in extending the shelf life of foods.

Efforts towards identifying the safe and natural sources of anti-oxidants of plant origin have notably increased in recent years. Compounds obtained from natural sources such as grain, oilseeds, honey, fruits and vegetables have been investigated for their natural anti-oxidant effects in meat products. Anti-oxidant effects of

cherry fruits in pork patties and sausages were researched by Britt, Gomma, Gray, and Booren (1998). Use of apple as an anti-oxidant in sausages has been reported by Osada, Hoshina, Nakamura, and Sugano (2000). Citrus fruits by-products have been investigated for their use in meat products (Fernandez-Lopez et al., 2004). Anti-oxidant effect of green tea leaves extracts in turkey sausages was reported by Bozkurt (2006). Sensory attributes and phenolic content of precooked pork breakfast sausage with fruit purees was investigated by Leheska, Boyce, Brooks, Hoover, Thompson, and Miller (2006). Addition of bearberry in pork products improved oxidation stability (O'Grady, Carpenter, Lynch, O'Brien, & Kerry, 2008). Similar anti-oxidant effects of grape seed extracts in chicken thigh meat had been investigated by Brannan (2008). Anti-oxidant effects of grape seed, oregano extract and rosemary in frozen vacuum packaged beef and pork was evaluated by Rojas and Brewer (2008). Very recently Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores, and Guerrero Legarreta (2009) evaluated the anti-oxidant effect of rosemary and oregano extracts on TBARS and colour of model raw pork batters.

Pomegranate (*Punica granatum*) is native from Iran to northern India and cultivated over the whole Mediterranean region. Pomegranate rind is an inedible part/by-product obtained during processing of pomegranate juice. It is further reported that pomegranate rind is rich source of tannins and other phenolic compounds (Ozkal & Dinc, 1994). Recently use of pomegranate juice and rind powder as a source of natural anti-oxidant in chicken

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patties had been investigated (Naveena, Sen, Kingsly, Singh, & Kondaiah, 2008).

Kinnow or Tangerine (*Citrus reticulata*) is a citrus fruit variety grown in north Indian states, mainly Punjab and Rajasthan. During the winter months it is processed into juices by the industry and fruit vendors. In the process of juice extraction, 30–34% of kinnow peel is obtained as a major processing by-product. Kinnow peel is also a rich source of Vitamin C, carotenoids, and polyphenolic anti-oxidants, (Anwar, Naseer, Bhanger, Ashraf, Talpur, & Aladededune, 2008).

Thus, by-products from fruit processing can offer a practical and economic source of natural anti-oxidants that could replace synthetic anti-oxidants. Hence, the present study was envisaged to investigate the anti-oxidant properties of extracts of kinnow rind powder (KRP), pomegranate rind powder (PRP) and pomegranate seed powder (PSP) in cooked goat meat patties.

2. Material and methods

2.1. Material

Fresh thigh meat of goats (slaughtered at an age about 1.5–2 year and weighing 18–20 kg) was obtained from local retail meat processing plants. Meat was stored at 4 °C for approximately 4 h before use. Fresh meat samples were obtained separately for each of the replications. Fresh kinnows (*Citrus reticulata*) and pomegranate (*Punica granatum*) were also obtained from retail fruit market. Standard tannic acid (SD Fine Chemicals, Mumbai, India), Thiobarbituric acid (MP Biomedicals Pvt. Ltd., Mumbai, India), 1, 1, 3, 3-tetraethoxypropane (Sigma Aldrich, New Delhi, India) and 1,1-diphenyl -2-picrylhydrazyl (Sigma Aldrich, New Delhi, India) used in the study were of analytical grade.

2.2. Preparation of powders and extracts

Mature and healthy kinnow and pomegranate fruits were washed, cut manually and peeled off. The rind (peel) thus obtained cut into small pieces using a sharp knife and dried in an air circulatory tray drier (Narang Scientific Works, New Delhi, India) at 60 °C for 48 h. Dried pieces were cooled and powdered in a heavy duty kitchen grinder and sieved using a sieve, ASTM No. 10 (1.651 mm) and packed into 100 g units and stored at room temperature in high density polyethylene bags until extraction. About 10 g of dried rind powder was mixed with 100 ml boiled distilled water and left for 1 h. The extract obtained by filtration was analyzed for total phenolic content, DPPH radical scavenging activity and also incorporated in goat meat patties. Freshly prepared extract was used for each replication.

Similarly powder from pomegranate seeds was prepared by drying the pomegranate fruit seeds in a tray drier and grinding in a heavy duty kitchen grinder and sieving (using a sieve ASTM No. 10).

2.3. Preparation of goat meat patties

About 2 kg of thigh meat was minced twice (10 mm plate followed by 8 mm plates using a meat mincer (INALSA, India). After mincing the samples were assigned to one of the following four treatments. I. Control (meat without any natural anti-oxidant); II. KRP (10 ml extract of kinnow rind powder); III. PRP (10 ml extract of pomegranate rind powder) and IV. PSP (10 ml extract of pomegranate seed powder. Sodium chloride (2% w/w) and refined sunflower oil (5% w/v) was added to all the samples. The volume of KRP, PRP and PSP extracts were replaced with distilled water in control sample. Immediately after adding all ingredients samples

were thoroughly hand-mixed. Minced goat meat (70 g portions) was formed into patties and cooked in a hot air oven (Narang Scientific Works, New Delhi, India) until the internal temperature reached 80 °C (approximately 15 min at 170 °C). After cooling to room temperature the patties were aerobically packaged in low density polyethylene bags and stored at 4 ± 1 °C for 12 days and analyzed for total phenolic content, instrumental color, sensory attributes and thiobarbituric acid reactive substances (TBARS). All the parameters and sensory evaluation were evaluated on first day of storage and TBARS values were analyzed throughout the storage period at an interval of 3 days.

2.4. Analysis of samples

2.4.1. DPPH radical scavenging activity

The ability to scavenge 1,1-diphenyl -2-picrylhydrazyl (DPPH) radical by KRP PRP and PSP was estimated by the method of Singh, Murthy, and Jayaprakasha (2002). Extracts of KRP, PRP and PSP (100 µg) diluted with 0.1 M Tris-HCl buffer (pH 7.4) was mixed with 1 ml of DPPH (250 µM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and then absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Model: Spectroscan 80 DV Biotech Eng. Management Company Ltd., UK). The scavenging activity was calculated by the following equation:

$$\text{Scavenging activity}\% = \frac{(\text{Absorbance}_{\text{Blank}} - \text{Absorbance}_{\text{Sample}})}{(\text{Absorbance}_{\text{Blank}})} \times 100$$

2.4.2. Total phenolics

Extracts of KRP, PRP and PSP and cooked goat meat patties (5 g of cooked patty was homogenized with 25 ml of 70% acetone and kept overnight for extraction at refrigeration temperature) were analyzed for total phenolics content using the Folin-Ciocalteu (F-C) assay (Negi & Jayaprakasha, 2003; Naik, Jayaprakasha, & Singh, 2008). Suitable aliquots of extracts were taken in a test tube and the volume was made to 0.5 ml with distilled water followed by the addition of 0.25 ml F-C (1 N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance recorded at 725 nm after 40 min. The amount of total phenolics was calculated as tannic acid equivalent from the calibration curve using 0.1 mg/ml of standard tannic acid solution (SD Fine Chemicals, Mumbai, India).

2.4.3. pH of goat meat patties

The pH of cooked patties was determined by blending 10 g sample with 50 ml distilled water. The pH values were measured using an electrode attached to a digital pH meter (Deluxe pH meter model-101E, Electronics India). The electrode was standardized against standard pH solutions of 4 and 8.

2.4.4. Instrumental colour evaluation

Effect of addition of extracts of natural anti-oxidant on colour properties of cooked goat meat patties were evaluated by Hunter Colorimeter (Hunter & Harold, 1987). Colorimetric analysis on freshly cooked goat patties was performed using a Hunter Lab Miniscan XE Plus colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) with a 25 mm aperture set for illumination D65, 10° standard observer angle. Hunter *L* (lightness), *a* (redness) and *b* (yellowness) values were measured on the outer and inner surfaces of horizontally cut goat meat patties.

2.4.5. Thiobarbituric reacting substances (TBARS) value

Lipid oxidization was monitored by measuring thiobarbituric acid reactive substances at an interval of 3 days during storage.

TBARS were determined using extraction method described by Witte, Krauze, and Bailey (1970). TBARS were extracted in chilled 20% trichloroacetic acid. Thiobarbituric extracts of each sample were used for measuring the absorbance at 520 nm. 1, 1, 3, 3, tetraethoxypropane (Sigma Aldrich, New Delhi, India) was used as standard for TBARS assay. TBARS numbers were calculated as mg of malonaldehyde per kg of meat.

2.4.6. Sensory evaluation

A semi trained panel of 6–8 members evaluated the cooked goat meat patties. The panelist rated each sample for colour and appearance, flavour and overall acceptability on six point descriptive scale (Keeton, 1983). Patties were pre warmed before serving and water was served for rinsing the mouth between samples.

2.4.7. Statistical analysis

Three replications of the study were performed and measurements of all parameters were made in duplicate. Mean values for various parameters were calculated and compared by analysis of variance using the SPSS software for windows (version 13.0). Means of pH, DPPH, total phenolics and hunter colour values and sensory attributes were analyzed using one-way ANOVA. Storage data of TBARS values were analyzed using two-way ANOVA with treatment and storage time as main effects. Statistical significance was identified at the 95% confidence level ($P < 0.05$). The average values were reported along with standard deviation (\pm Standard Deviation).

3. Results and discussion

The DPPH free radical scavenging activity (DPPH%) and total phenolic content ($\mu\text{g/g}$ powder) are illustrated in Figs. 1 and 2, respectively. All the extracts of powders showed an excellent ability in radical scavenging activity (30.50–50.50%) and higher content of total phenolics (2470–5851 $\mu\text{g/g}$). Among three extracts, PRP had significantly ($P < 0.05$) higher amount of phenolics followed by KRP and PSP. In contrast PSP showed significantly ($P < 0.05$) greater free radical scavenging activity followed by KRP and PRP. Negi and Jayaprakasha (2003) have also reported radical scavenging activity of PRP. Similar to these findings Naveena et al. (2008) reported free radical scavenging activity in juice extract of pomegranate seeds and rind powder.

Results of effect of fruit powders on pH and phenolic content of cooked goat patties is presented in Table 1 and Fig. 3, respectively. The pH was significantly ($P < 0.05$) lower in KRP and highest in control. Furthermore pH of the cooked patties decreased due to addition of extracts. This may be attributed to the acidic pH of the fruit extracts. KRP patties had the lowest pH followed by PSP & PRP. In contrast, Naveena et al. (2008) reported no significant dif-

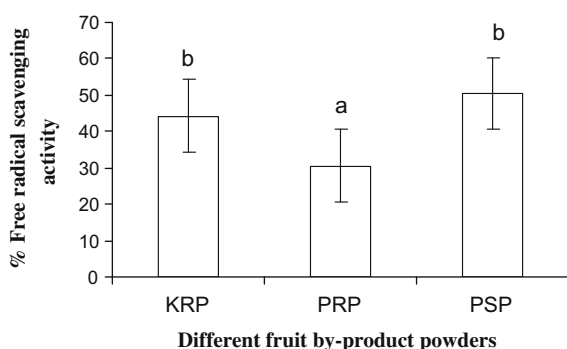


Fig. 1. The DPPH radical scavenging activity (%) of extracts of kinnow rind powder (KRP), pomegranate rind powder (PRP) and pomegranate seed powder (PSP).

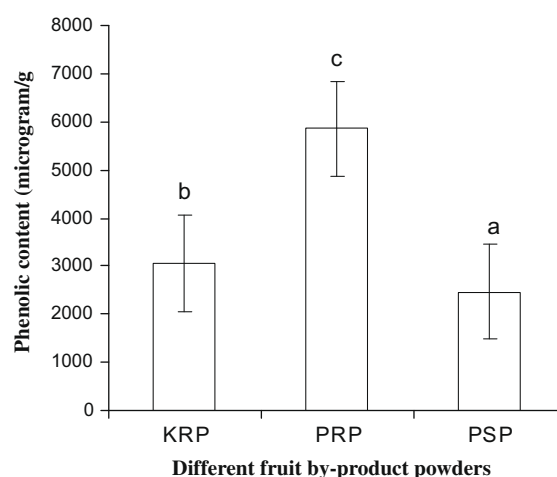


Fig. 2. Total phenolics content ($\mu\text{g/g}$) of kinnow rind powder (KRP), pomegranate rind powder (PRP) and pomegranate seed powder (PSP).

ference in pH of cooked chicken patties due to addition of pomegranate juice extract.

Total phenolic content ($\mu\text{g/g}$) was significantly ($P < 0.05$) higher in PRP followed by KRP and PSP. Similarly Leheska et al. (2006) observed an increase in phenolic content of precooked pork breakfast sausages prepared with fruit purees. Naveena et al. (2008) have also reported that PRP significantly increased the total phenolic content of cooked chicken patties.

Instrumental colour evaluation (Table 1) revealed a significant ($P < 0.05$) effect of extracts on Hunter colour values. Addition of KRP extract increased L values whereas PRP extract reduced L values. However, control and PSP treated patties did not show any significant difference for L value. Hunter Lab 'a' values (redness) were significantly reduced in PRP and PSP patties as compared to control and KRP treated patties. Yellowness (b value) significantly ($P < 0.05$) reduced due to addition of PRP, whereas no significant difference was observed between control, KRP and PSP patties. Similar to these observations, Naveena et al. (2008) reported that addition of PRP reduced the lightness (L) value. However they have further found an increase in redness value of chicken patties due to addition of PRP. The dark colour of PRP might be responsible for decrease in L value in PRP treated goat meat patties.

In contrast to instrumental colour values, sensory evaluation for colour and appearance did not reveal any significant difference among the samples. Furthermore panelists did not find any significant difference for flavour and overall acceptability of different type of patties. Thus all the products were equally acceptable. It was further observed that addition of extracts of KRP, PRP and PSP did not have any negative effect on sensory attributes of goat meat patties.

Effect of KRP, PRP and PSP on TBARS values during refrigerated storage of goat meat patties are shown in Figs. 4 and 5. All the treatments significantly ($P < 0.05$) reduced the TBARS values throughout storage as compared to control. The lipid oxidation reduction was highest in PRP as compared to PSP and KRP. TBARS values significantly ($P < 0.05$) increased in control throughout storage. However in KRP and PSP patties TBARS increased only up to 6th day of storage. It is interesting to observe that TBARS remained constant through out the storage period in PRP indicating very strong anti-oxidant effect. The data further indicated the marked anti-oxidant activity of KRP, PRP and PSP. Observations on percent reduction of TBARS formation by different powders indicated that greater reduction of TBARS by PRP (67%), followed by PSP (40%) and KRP (36%). However, the percentage increase in TBARS was

Table 1
pH, Hunter color values and sensory scores of different goat meat patties.

Treatments	pH	L value	a value	b value	Colour and appearance	Flavour	Overall acceptability
Control patties	6.23 ± 0.02 ^d	38.99 ± 1.62 ^{bc}	8.42 ± 0.55 ^b	18.68 ± 1.46 ^b	5.41 ± 0.15	5.58 ± 0.15	5.68 ± 0.22
KRP patties	6.02 ^a ± 0.07	40.37 ± 2.22 ^c	8.64 ± 0.37 ^b	15.45 ± 2.68 ^b	5.50 ± 0.18	5.66 ± 0.25	5.65 ± 0.25
PRP patties	6.15 ± 0.02 ^c	26.80 ± 1.27 ^a	7.03 ± 0.33 ^a	10.40 ± 0.42 ^a	5.41 ± 0.37	5.66 ± 0.10	5.50 ± 0.13
PSP patties	6.09 ± 0.01 ^b	37.49 ± 1.10 ^b	7.78 ± 0.29 ^a	16.44 ± 0.66 ^b	5.50 ± 0.13	5.58 ± 0.15	5.58 ± 0.08

Number of observations: six for pH; sixteen for lightness (L), redness (a), yellowness b and 18 observations for sensory scores.

Mean values in the same column bearing the same superscripts do not differ significantly ($P < 0.05$).

Sensory scores are measured on 6 point descriptive scale where 1 = extremely poor and 6 = highly acceptable.

KRP-Kinnow rind powder extract. PRP-Pomegranate rind powder extract. PSP-Pomegranate seed powder extract.

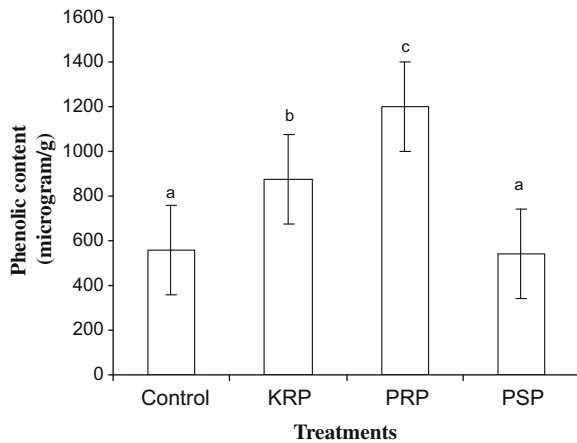


Fig. 3. Total phenolics content (µg/g) of goat meat patties containing the extracts of kinnow rind powder (KRP), pomegranate rind powder (PRP) and pomegranate seed powder (PSP).

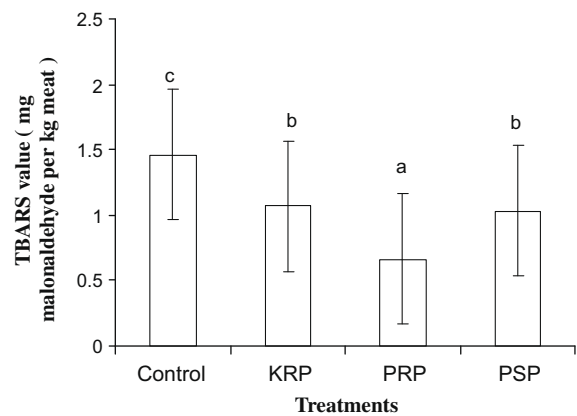


Fig. 4. Overall treatment means of TBARS values (mg malonaldehyde/kg meat) of goat different goat meat patties during refrigeration storage at 4 ± 1 °C.

highest in control (200%), followed by PSP (125%), KRP (44%) and least in PRP (only 2%).

Observation of over all changes on lipid oxidation during 12 days of refrigerated storage revealed that TBARS values reached its threshold limit of (2 mg/kg meat) on 9th days in control patties. TBARS values of patties treated with extracts remained below the acceptable level of 2 mg/kg meat during 12 days of storage. Regardless of treatment, TBARS gradually increased during 12 day storage. Similarly regardless of storage period, TBARS was lowest in PRP followed PSP, KRP and highest in control. A critical observation on average TBARS values during 12 days of storage

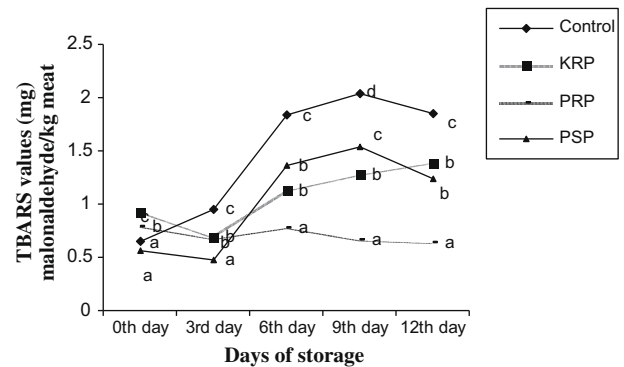


Fig. 5. Antioxidant effect of extracts of kinnow rind powder (KRP), pomegranate rind powder (PRP) and pomegranate seed powder (PSP) in goat meat patties during refrigeration storage at 4 ± 1 °C.

suggested that among all the treatment TBARS was in the order PRP < PSP < KRP < Control indicating significant anti-oxidant effects of the extracts of powders used in the study.

A significant relation between phenolic content and anti-oxidant effect of pomegranate peel extract has been reported by Negi and Jayaprakash (2003). Similarly Li et al. (2006) observed strong anti-oxidant effect of PRP and pulp. Naveena et al. (2008) found that PRP reduced the TBARS values in chicken patties. Rojas and Brewer (2008) reported relationship between phenolic content & anti-oxidative effect of grape seed extract in beef and pork. Brannan (2008) reported anti-oxidant effect of grape seed extract in chicken patties. Even though the phenolics compounds reported to be highly responsible for the anti-oxidant activity in fruits, possible synergism of phenolic compounds might be responsible for these observations.

4. Summary

A variety of anti-oxidant compounds, mostly phenolic in nature exist in fruits by-products. These can provide protection against lipid oxidation in high fat containing meat products. In this study use of extracts of KRP, PRP and PSP significantly reduced lipid oxidation in cooked goat meat patties. Incorporation of 10 ml extract of these powders (10 g/100 ml) could protect cooked goat meat patties against lipid oxidation during refrigerated storage. All individual extracts were effective to maintain low TBARS values in cooked goat meat patties. Extract of PRP was more effective in reducing TBARS formation. It was concluded that extracts of these powders could be successfully added to meat to function as anti-oxidant. It is important to note that while processing kinnow & pomegranate into juice, the rind & pulp are discarded. The food industry can make use of these by-products as source of natural anti-oxidants in processed meat products. Further research is required to identify, isolate and quantify the specific bio- active anti-oxidant compounds from these fruit by-products.

5. Implications

It is encouraging to observe that extracts of kinnow and pomegranate fruits by-products were having anti-oxidative effect in goat meat patties. Anti-oxidant characteristics of these fruit by-products could provide scientific basis for using these fruit by-products in meat products. As promising as these results are, additional research will be required to determine how these powders could be used as health promoting functional ingredients in meat processing.

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