

Original Research Article

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Extraction of Total Polyphenols (TPP) from Mango Seed Kernels and its Incorporation in Watermelon Squash

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ABSTRACT

Processing of fruits generates its seeds and peels as wastes. Disposal of these materials in the environment usually represents a problem that is further aggravated by legal restrictions. Seeds of mango fruit generated as waste during its processing. In the present study attempt has been made to utilize this waste as by product to extract total polyphenols (TPP), which are one of the functional ingredient from plant sources and known to exhibit various health benefits. Seed wastes of Totapuri variety mango fruits were collected and kernels were separated from seed coat. Further these kernels were processed into flour and utilized for extraction of total polyphenols (TPP). Extracted TPP analysed for its antioxidant and antimicrobial property, then incorporated in watermelon squash. After incorporation squash samples were tested for various physico-chemical and sensory properties. Results revealed that, mango kernel flour (MKF) had 47.50 mg GAE/gm TPP. Hundred grams of MKF yielded 4.25 g of TPP extract in aqueous form. The concentration of TPP water extract was 21.25 mg GAE per ml. Antioxidant activity of one gram of TPP extract was equivalent to 625 mg of vitamin C and scavenged 71.42 per cent DPPH radicals. TPP extract was most effective against *Bacillus magatirius* compared to other microbial species tested for antimicrobial property indicated by zone of inhibition of 24mm. As the level of incorporation of TPP extract increased in watermelon squash its antioxidant activity was also observed to be significantly increased. Out of incorporation levels 0.1, 0.25 and 0.5 per cent, 0.1 per cent TPP incorporated squash sample had sensory scores on par with control and observed to be acceptable by panel members.

Keywords

Total Polyphenols (TPP),
Mango Seed
Kernels,
Watermelon
Squash.

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Introduction

Mango (*Mangifera indica*) is the main fruit of Asia and has its own importance all over the world. Mango continues to dominate the Indian fruit basket contributing 36 per cent to total fruit area and 20.3 per cent to total fruit production (Kusuma and Basavaraja, 2014). Therefore strategies for the profitable use of these materials are needed. In the fruit processing industry, edible portions of fruits are processed into products such as puree, canned slices, juice and pickles, whereas

seeds and peels are often will be discarded as waste. A disposal of these materials usually represents a problem that is further aggravated by legal restrictions. Thus, new aspects concerning the use of these wastes as by-products for further exploitation on the production of food additives or supplements with high nutritional value have gained increasing interest because these are high-value products and their recovery may be economically attractive. It is well known that

by-products represent an important source of sugars, minerals, organic acid, dietary fibre and phenolics (Jasna, 2009). Mango seed kernel was shown to be a good source of phenolic antioxidants, metal chelators and tyrosinase inhibitors (Maisuthisakul and Phasuk, 2009). Abdalla *et al.*, (2006) characterized the phenolic compounds in Egyptian mango seed kernels. The components included tannins, gallic acid, coumarin, ellagic acid, vanillin, mangiferin, ferulic acid, cinammic acid and unknown compounds. Antioxidant effect of the mango seed kernel was due to its high content of polyphenols, sesquiterpenoids, phytosterols (Ashoush and Gadallah, 2011).

With this background in the present study was undertaken on mango seed kernels which generated as industrial waste are collected and used as by product to extract Total Polyphenols (TPP). Extract was studied for its antioxidant and antimicrobial activities. Further this TPP extract was incorporated into watermelon squash with the objective to enhance its antioxidant activity and to study its acceptability level.

Materials and Methods

Procurement of mango seed sample

Seed waste of Totapuri variety fruit was procured from a local mango processing, Safal industry, Bengaluru, Karnataka, India.

Processing of mango seed into kernel flour (Ashoush and Gadallah, 2011)

Mango seeds were washed and dried in hot air at 60° C for 3-4 hours. Mango kernels were separated from stone manually using stainless steel knife. Kernels were soaked (6-7 hrs), blanched (1-2 min), chopped, dried and ground into flour, sieved and stored in air tight container.

Estimation of total polyphenols (Sadasivam and Manickam, 1991)

Blue colour developed by polyphenols with FCR reagent in alkaline condition was measured at 650 nm (Annexure XIV). In the present study polyphenol content of whole mango kernel, which was directly powdered in electric blender without application of any processing conditions was analyzed, along with processed mango kernel flour (MKF).

$$\frac{\text{mg/ 100gm sample total polyphenols}}{\text{Concentration of polyphenol from graph}} = \frac{\text{Aliquot taken for Estimation}}{\text{Weight of Sample}} \times 5 \times \frac{100}{100} \times \frac{1}{100}$$

Extraction of total polyphenols from mango kernel flour

Hundred gram of mango kernel flour was extracted with 1000ml of 80% acetone for 4-5hr by stirring with magnetic stirrer. The slurry was then strained through muslin cloth to separate the extract.

The total polyphenols extract was then subjected to Rotary evaporation at 45°C to remove acetone completely and to obtain concentrated extract in liquid form. This was stored in air tight container and kept in cool place (Sadasivam and Manickam, 1991).

Estimation of antioxidant activity by DPPH method

The antioxidant activity was expressed in terms of ascorbic acid equivalents, so ascorbic acid was taken as substrate. Various concentrations of ascorbic acid were prepared and added to DPPH solution.

The decrease in O.D is plotted against concentration of ascorbic acid. The concentration of sample was calculated using the standard curve.

Assessment of antimicrobial activity of total polyphenol (TPP) extracts (Kaur *et al.*, 2010)

Three bacterial strains (*Bacillusmagatirius*, *Staphylococcus aureus* and *Eshcertia coli*) were used in this study to test antimicrobial activity of TPP extract. The bacterial strains were grown in 50 mL of nutrient broth at 37° C. The disc diffusion technique was used for antimicrobial test. An overnight suspension culture of the three bacterial strains was spread on the nutrient agar media. Sterile discs were prepared and placed on the culture spread agar media. The discs were impregnated with the 50µL TPP extract of mango seed kernel. Distilled water was included as a negative control and 0.001 per cent mercuric chloride was included as a positive control to determine the sensitivity of the bacterial strains. The inoculated plates were incubated at 37° for 24 h. The antibacterial activity was evaluated by measuring diameter of the inhibition zone around the disc.

Incorporation of TPP extract into watermelon squash

Watermelon fruit contains low total polyphenols (Ref). Hence this fruit was selected to prepare squash and incorporate total polyphenols (TPP) extract at 0.1 (100 mg), 0.25 (250 mg), and 0.5 (500 mg) per cent levels and compared with control-1 (with preservative Sodium Benzoate) and control -2 (without any preservative). The squash prepared was diluted in the ratio of 1: 4 ratio with water and served to panellists for sensory evaluation.

Analysis of physicochemical characteristics of squash

Total soluble solids (TSS)

The Homogenized squash sample was used to

measure the TSS. It was determined by hand refractometer. For each entry, replicated thrice and the mean was expressed in °Brix after temperature corrections.

pH

pH of the squash sample was determined using digital pH meter.

Total sugars (Sadasivam and Manickam, 1991)

A known quantity of filtrate was hydrolysed by mixing with 5 ml of hydrochloric acid and kept overnight. Then, it was neutralized with sodium hydroxide using phenolphthalein as indicator and diluted suitably. An aliquot was taken, total sugar was estimated using Shaffer-Somogyi method and recorded as per cent

Titration acidity (Ranganna, 1986)

A suitable aliquot was titrated against standard 0.1 N sodium hydroxide solution using phenolphthalein as indicator. Results were expressed as per cent citric acid. Total polyphenol content and antioxidant activity of squash were also analysed.

Organoleptic evaluation of value added products

All the watermelon squash samples were evaluated by a panel of semi-trained panel (n=21). The products were evaluated for appearance, consistency, taste, colour, aroma and overall acceptability on nine point hedonic scales (Avantina, 2006).

Statistical analysis (Fisher and Yates, 1963)

The data was tabulated keeping in view the objectives of the study. One way analysis of variance (F-test) was applied for sensory

analysis and for physicochemical properties of squash, to test the significant difference between samples in the study. Student t- test was done to compare antimicrobial properties of samples. Significant difference was defined at $p \leq 0.05$.

Results and Discussion

Procurement and processing of Mango Seed Kernel

Per cent distribution of mango fruit parts was studied and depicted in Fig. 1. Mango seed coat and kernel together represent 14.9 per cent of the whole mango fruit weight, out of which mango kernel alone represents 9 per cent. Kernels were separated manually from seed coat and subjected for some simple processing steps to convert it into flour. Throughout these processing steps weight of the sample varies due to uptake and evaporation of water. Flour recovered from mango kernels was 80.6 per cent.

Total polyphenol content of Mango Kernel Flour (MKF)

Polyphenol content of whole mango kernel and MKF was estimated and results are presented in Fig 2. Statistical analysis showed the significant difference between the samples tested for polyphenol content. Results indicate that whole mango kernel has significantly high polyphenol content (56.50 mg GAE/gm) compared to MKF (47.50 mg GAE/gm). But converting whole kernels directly into powder form without any processing steps was found to be difficult.

Hence some simple processing methods were employed to soften the kernels and to convert it into powder form to obtain MKF. The same was utilized for the extraction of total polyphenol (TPP) instead of whole kernels even if it was observed high in TPP content.

Extraction of total polyphenols from MKF and antioxidant activity

Hundred grams of MKF yielded 4.25 g of TPP extract in aqueous form. The concentration of TPP water extract was 21.25 mg GAE per ml. Antioxidant activity of whole mango kernel, its processed flour and TPP extract are presented in Table 1. Antioxidant activity was expressed in terms of mg of vitamin C equivalents per gram of sample and also in terms of per cent radical scavenging activity exerted by 500 μ g of sample. It was found to be highest for one gram of total polyphenol extract, equivalent to 625 mg of vitamin C and scavenged 71.42 per cent DPPH radicals. Antioxidant activity of whole mango kernel and MKF was 105 and 60.5 mg of vit C equivalents per g of sample respectively. For the same samples per cent radical scavenging activity was found to be 63.40 and 58.92 per cent respectively.

Antimicrobial property of TPP extract

Antimicrobial property was tested against 3 strains i.e. *Bacillus megatirius*, *Staphylococcus aureus* and *Eshcertia coli*. Results were compared with the 0.001 per cent mercuric chloride as positive control, which is used as common antimicrobial compound and distilled water was used as negative control, which does not possess any antimicrobial activity.

Distilled water has not developed any zone of inhibition against all the three tested microbial strains. Hence inhibition zone developed by TPP extract and mercuric chloride were used in table for statistical comparisons. Statistically significant difference existed between antimicrobial properties of control and TPP extract on microbial species tested. TPP extract against *Bacillus magatirius*, exhibited more zone of inhibition compared mercuric chloride. Zone

of inhibition against *Bacillus magatirius* was 24 and 19 mm for TPP extract and mercuric chloride respectively. In case of *Staphylococcus aureus*, mercuric chloride showed more zone of inhibition (24mm) compared to TPP extract (19mm). Zone of inhibition developed against *E. coli* by mercuric chloride and TPP extract was 20 and 16 mm respectively (Table 2) (Fig.3).

Incorporation of TPP extract into watermelon squash

Watermelon squash was developed with the incorporation of TPP (Total polyphenol) extract at, 0.1 (100mg), 0.25 (250mg), 0.5 (500 mg) per cent levels per 100 ml of squash. Samples without TPP extract incorporation were considered as control. Procedure followed for the preparation of squash is presented in Fig.4.

Physico-chemical properties of watermelon squash

Physico-chemical properties of watermelon squash were studied and results are presented in Table 3. TSS (Total Soluble Solids) per cent significantly varied between control and TPP extract incorporated squash. Both the control squash samples (1 and 2) had lower TSS i.e 51.3 and 51.6 per cent. As the level of TPP extract incorporation increased, TSS per cent also increased. TSS for 0.1, 0.25 and 0.5 per cent TPP extract incorporated squash samples was, 55.3, 56.9 and 57.2 per cent respectively.

Squash samples were tested for pH and observed to be significantly different between the control and TPP extract incorporated squash. Control samples had pH of 3.08 (control-1) and 3.45 (Control-2). TPP extract incorporated samples had pH of 3.25 (0.1%), 3.14 (0.25%) and 3.10 (0.5 %). As the level of TPP incorporation increased pH was decreased.

TPP extract incorporated samples had more total sugar values compared to control samples. Total sugar values were found to be, 41.70 (Control- 1) and 40.19 (Control- 2). For TPP extract samples it ranged between 46.67 to 62.94 per cent. Significant difference was observed for total sugar content of different squash samples.

Titration acidity was found to be more for control- 1 (1.50%) followed by control- 2 (1.25 %). Among the TPP extract incorporated samples, 0.5 per cent incorporation level had more titration acidity (1.20 %), followed by 0.25 (1.03 %) and 0.1 per cent (0.83 %) incorporation levels. As the level of TPP incorporation increased, titration acidity was increased. Difference in titration acidity among squash samples was observed to be statistically significant.

Total polyphenol content and antioxidant activity significantly differed between control and TPP extract incorporated squash samples. As the level of TPP extract incorporation increased, there was also increase in polyphenol content (115 to 512 mg GAE/100ml) and antioxidant activity. Total polyphenol content of 10.0 and 12.0 mg GAE/100 ml was observed for control- 1 and 2 respectively.

Antioxidant activity was lowest for control-1 (6.25 mg Vit C eq./100 ml) and highest for 0.5 per cent TPP (320 mg Vit C eq./100 ml) (Fig 5).

Sensory evaluation of watermelon squash

Sensory evaluation of watermelon squash developed by incorporation TPP extract was conducted and results are presented in Table 4. Results showed statistically significant difference between control and TPP extract incorporated watermelon squash samples for all the sensory attributes. Incorporation level

of 0.1 per cent TPP into squash exhibited sensory scores on par with control (both 1 and 2), whereas further incorporation significantly decreased the scores. Both the control samples had higher scores for all the sensory attributes compared to TPP extract incorporated samples. Among the TPP extract incorporated samples 0.1 per cent level of incorporation was found to be best accepted with the scores of, 8.15 (appearance), 8.20 (consistency), 8.10 (colour), 8.09 (aroma), 8.10 (taste) and 8.13 (overall acceptability), whereas 0.25 and 0.5 per cent level had significantly lower sensory scores.

The percentage distribution of mango fruit parts depict that, kernel along with seed coat (outer hard seed coat) represents 14.9 per cent and kernel alone accounts for 9.0 per cent of the total fruit weight. Results obtained were in agreement with the findings of Dhingra and Kapoor (2007) reported, seed and kernel comprised 18 and 10 per cent of total fruit, respectively. Elegbede *et al.*, (1995) reported that kernel constitutes 10 per cent of the fruit weight. Variations in seed and kernel per cent of the mango fruits is attributed to the varietal difference.

Phenolic compounds are widely distributed in all plants, the most common polyphenol classes being phenolic acids, flavonoids, and tannins. These compounds constitute a very diverse group of secondary plant metabolites (Ovaskainen *et al.*, 2014). Epidemiological studies and associated meta-analyses strongly suggest that long term consumption of plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Manach *et al.*, 2014).

Total polyphenol content of MKF was 4.75 g per 100 g of flour (47.5 mg GAE per g. Lower value was quoted by Ashoush and

Gadallah (2011) and Norshazila *et al* (2010), reported 23.90 and 32 mg GAE per g respectively. Total amount of TPP extracted was 4.25 g (in 200 ml aqueous form) per 100 g of MKF.

In whole mango kernels total polyphenol was found to be 56.5 mg GAE per g. Higher values were reported by Soong and Barlow (2004) i.e. 117 mg GAE per g this variation may be attributed to varietal difference. In MKF it was observed to be low compared to whole mango kernel (47.5 mg GAE per g), which may be due to loss of certain water extractable polyphenolic compounds during processing of MKF (observed through the colour change of water during soaking and blanching). Lower TPP content of MKF was reported by Bandyopadhyay *et al.*, (2014) i.e. 27.9 mg GAE per g. Which indicate processing methods have impact on TPP content of mango kernel flour.

TPP extract was obtained in aqueous solution, in the concentration of 21.25 mg GAE per ml, which represent its polyphenol content. Khammuang and Sarnthima (2011) reported 399.8 mg GAE/g of extract, where it was lyophilised and concentrated.

In the present study highest antioxidant activity was observed for TPP extract. One gram of total polyphenol (present in 47 ml of extract) equivalent to the antioxidant activity possessed by 625 mg of vitamin C. Aliquot containing 5 μ L of TPP extract expressed the radical scavenging activity of 71.24 per cent. Norshazila *et al.*, (2010) reported 95.12 per cent, for the extract with concentration 1mg per ml.

An aliquot containing 500 μ g of whole mango kernel sample showed 63.40 per cent of radical scavenging activity. Ashoush and Gadallah (2011) reported that, mango kernel flour has the radical scavenging activity of

95.08 per cent but in present study it was found to be lower i.e. 58.92 per cent. This difference may be due to the difference in variation of polyphenols content of samples due to processing methods employed. According to Khammuang and Sarnthima (2011), antioxidant activity has a positive correlation with mango seed extracts total phenol content. In the present study results of antioxidant activity indicate that, TPP extract exhibit strong antioxidant activity.

Physico-chemical properties of watermelon squash

Total soluble solids (TSS) of water melon squash ranged between, 51.3 to 57.2 per cent. As the level of incorporation of TPP increased TSS per cent also increased due increase in soluble molecules. Similar values 51.50 and

51.10 per cent of TSS were reported by Malik *et al.*, (1994) for watermelon squash containing sodium benzoate and potassium metabisulphite as preservative respectively.

In the present study pH of the watermelon squash ranged between 3.08 and 3.45 and was statistically significant. Use of citric acid in the preparation of squash was attributed to the lower pH of the samples. Similar values reported by Malik *et al.*, (1994) which were 3.0 and 2.95.

Total sugar content of watermelon squash samples ranged from 41.70 and 62.94 per cent. TPP extract incorporated squash samples had higher values for total sugar content compared to control samples. These results are in correlation with the study conducted by Relekar *et al.*, (2013).

Table.1 Antioxidant activity of mango kernel, MKF and TPP extract

Sample	Antioxidant activity (mg of Vit. C equivalents /g)	Per cent radical scavenging activity of 500 µg of sample
Mango kernel	105.0	63.40
MKF (Mango kernel flour)	60.5	58.92
Total Polyphenol extract	625.0	71.42
F - Test	*	*
SEm±	0.37	0.21
CD at 5% level	1.17	0.66

* Significant at 5% level

Table.2 Antimicrobial activity of Total Polyphenol (TPP) extract

Sample	<i>Bacillus megaterium</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Escherichia coli</i> (mm)
TPP extract	24.0±0.2	19.0±0.5	16.0±0.2
Mercuric Chloride (positive control)	19.0±0.1	26.0 ±0.5	20.0±0.5
t - Test	38.72*	17.14*	12.86*

Note: Distilled water (negative control) has not exhibited any zone of inhibition

* Significant at 5% level

Table.3 Physico-chemical properties of watermelon squash incorporated with TPP extract

Level of incorporation	TSS (%)	pH	Total sugars (%)	Titration acidity (%)	Total Polyphenol Content (mg/100m)	Antioxidant activity (mg vit C equivalents /100ml)
Control -1 (without preservative)	51.30 ^c	3.08 ^c	41.70 ^d	1.50 ^a	10.0 ^e	6.25 ^d
Control -2 (with preservative)	51.60 ^c	3.45 ^{aa}	40.19 ^e	1.25 ^b	12.4 ^d	7.75 ^d
TPP extract 0.1 %	55.30 ^b	3.25 ^b	46.67 ^c	0.84 ^e	115.0 ^c	71.80 ^c
TPP extract 0.25 %	56.90 ^a	3.14 ^c	58.28 ^b	1.03 ^d	258.0 ^b	161.25 ^b
TPP extract 0.5 %	57.20 ^a	3.10 ^c	62.94 ^a	1.20 ^c	512.0 ^a	320.0 ^a
F - Test	*	*	*	*	*	*
SEm±	0.20	0.02	0.04	0.01	0.70	0.56
CD at 5% level	0.65	0.06	0.12	0.03	2.17	1.77

* Significant at 5 % level (Means in the same columns followed by different letters are significant)

Table.4 Mean sensory scores of watermelon squash incorporated with TPP (Total Polyphenol) extract

Level of Incorporation	Appearance	Consistency	Colour	Aroma	Taste	Overall acceptability
Control- 1 (without preservative)	8.20 ^a	8.18 ^a	8.15 ^a	8.10 ^a	8.15 ^a	8.15 ^a
Control- 2 (with preservative)	8.21 ^a	8.20 ^a	8.18 ^a	8.12 ^a	8.13 ^a	8.18 ^a
TPP extract 0.1 %	8.15 ^a	8.20 ^a	8.10 ^a	8.09 ^a	8.10 ^a	8.13 ^a
TPP extract 0.25 %	7.90 ^b	8.02 ^a	7.72 ^b	7.80 ^a	7.60 ^b	7.73 ^b
TPP extract 0.5 %	6.95 ^c	7.22 ^b	6.70 ^c	7.00 ^b	6.52 ^c	6.53 ^c
F - Test	*	*	*	*	*	*
SEm±	0.15	0.12	0.15	0.14	0.15	0.14
CD at 5% level	0.43	0.35	0.42	0.40	0.43	0.39

* Significant at 5% level (Means in the same columns followed by different letters are significant)

Fig.1 Per cent distribution of mango fruit parts

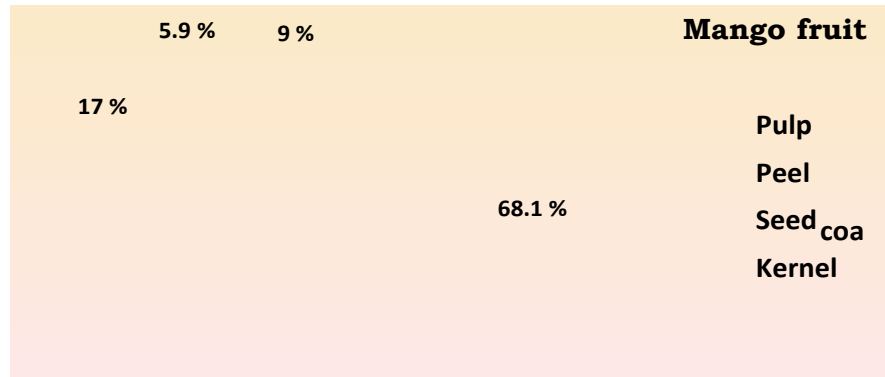


Fig.2 Total polyphenol content of mango kernel and MKF (mg of GAE/ g or ml)

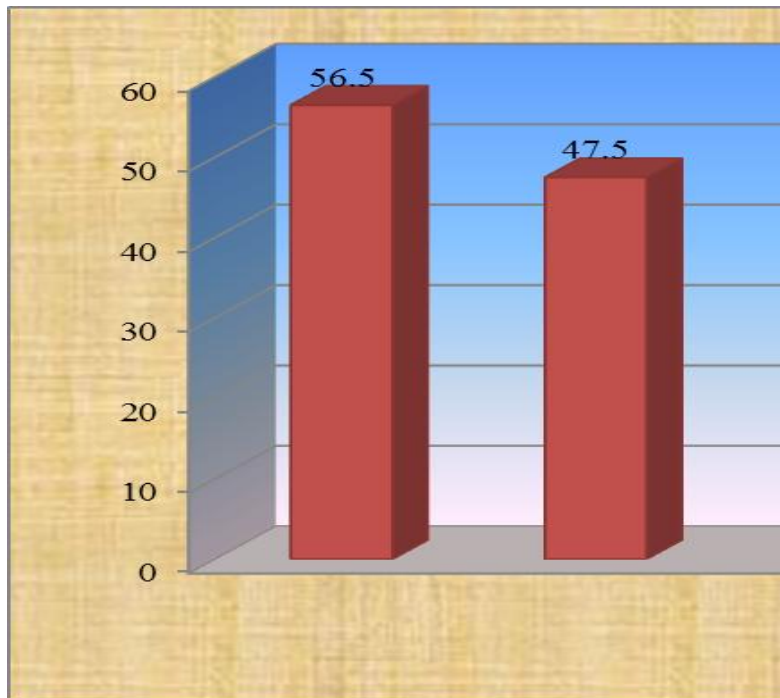


Fig.3 Antimicrobial activity of TPP extract

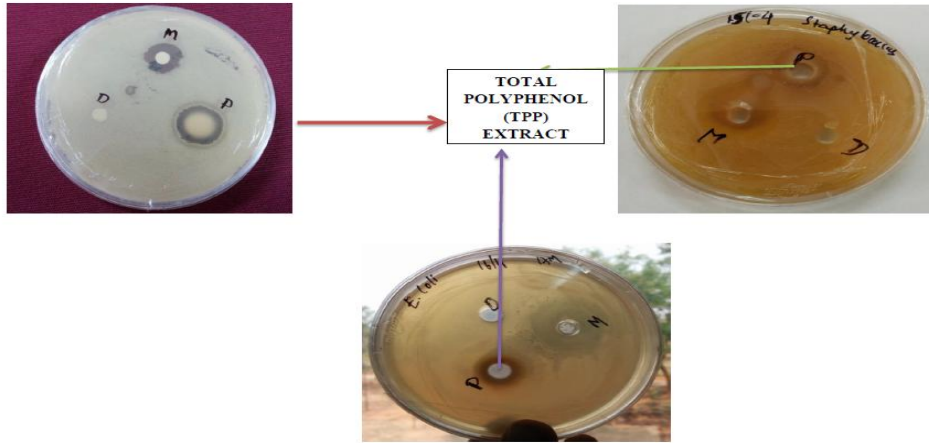


Fig.4 Preparation of watermelon squash

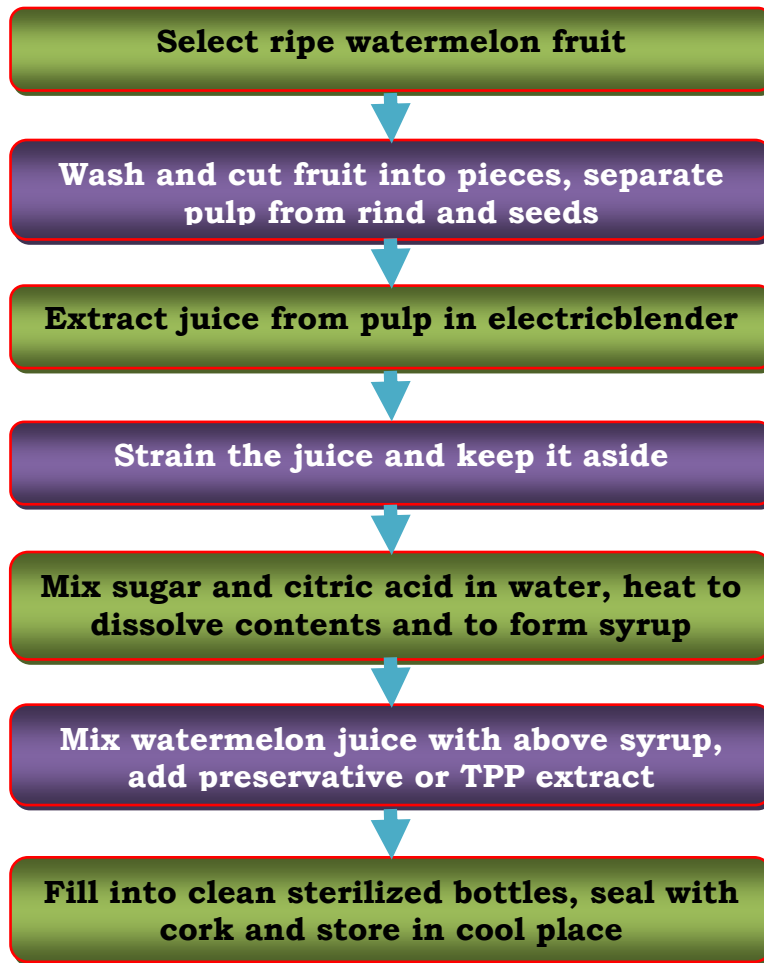
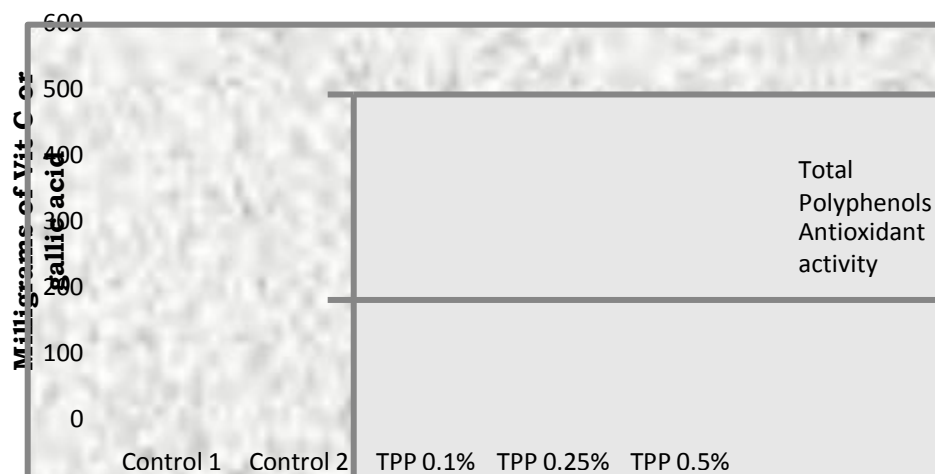


Fig. 4: Preparation of watermelon squash

Fig.5 Antioxidant activity (mg of Vit C eq. / 100 ml) of TPP incorporated watermelon squash



Titration acidity (TA) of TPP extract incorporated watermelon squash was found to be lower compared to both the controls. However, an increasing trend was observed in titration acidity of squash as the level of polyphenol incorporation increased (0.84 to 1.20 %). These findings are supported by Zarei *et al.*, (2010) who reported that titration acidity has a significant positive correlation with the total polyphenol content of pomegranate juice cultivars. The present study values of TA were in comparison with the results (0.83 to 0.93 %) reported by Swamy *et al.*, (2012).

Incorporation of total polyphenol extract significantly increased the polyphenol content of watermelon squash (115 to 512 mg per 100 ml) compared to control (10 to 12.4 mg per 100 ml). Findings of low polyphenol content of watermelon squash (without polyphenol incorporation) correlated with results (102.86 to 110.71 mg per kg) reported by Kim and Goodner (2009). In the present study the incorporation of TPP extract increased the antioxidant capacity of watermelon squash which exerts health benefits. Antioxidant activity of control squash samples (control- 1 and control- 2) was low compared to TPP extract incorporated samples exhibiting a positive correlation with the polyphenol content. The results are on par with the study revealed by Kaur *et al.*, (2010).

Sensory evaluation of watermelon squash

Both the control watermelon squashes (With and without preservative) scored high for all the sensory attributes. Scores obtained for 0.1 per cent TPP incorporated squash were on par with control and found to be accepted by panel members. Incorporation of TPP at higher levels imparted astringency taste due to the presence of tannins and resulted in a decrease of sensory scores.

In conclusion, seed kernels of mango fruit are generated as waste during its processing for various food products. These kernels can be utilized as a by-product to extract total polyphenols (TPP), as these functional compounds are known to exert various health benefits and possess antimicrobial activities. Mango seed kernels are good sources of polyphenols which possess strong antioxidant activity. Due to this antioxidant activity TPP extract exhibited antimicrobial activity against selected microbial strains. TPP extract when incorporated in watermelon squash, increased its antioxidant property. Due to astringent taste incorporation was acceptable at 1 per cent level in watermelon squash. There is also scope for further treatment and concentration of TPP extract and its study with respect to health benefits against lifestyle disorders.

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