Journal of Food Processing & Technology
ISSN: 2157-7110

Chauhan et al., J Food Process Technol 2014, 5:9
DOI: 10.4172/2157-7110.1000368

Research Article Open Access

Determination of Antioxidant Capacity, Total Phenolics and Antimicrobial Properties of Spray-Dried Guava Extract for Value-Added Processing

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Abstract

To explore the potential use of Guava as a functional food ingredient, aqueous guava extract was filtered to remove insoluble material; the soluble extract was spray-dried with the addition of 7% maltodextrin. Spray dried Guava concentrate showed increased levels of antioxidant capacity (2, 2-diphenyl-1-picrylhydrazyl DPPH assay 3.2 fold) and total phenolics (2.0 fold) but low levels of flavinoids. The addition of maltodextrin during spray-drying resulted in the formation of certain unresolved peaks analyzed by HPLC due to dilution effect, the redistribution of molecular weight is likely beneficial for in vivo absorption and increase its bioavailability. Spray-drying concentration also increased the proportion of ascorbic acid content (118.6 mg/100g) of guava powder and showed effective antimicrobial activity against *Shigella* (MIC 11mg/ml), *E coli* (MIC 8 mg/ml) and *Candida* spp. (MIC<1 mg/ml). The proximate and physiochemical properties showed good quality and increased solubility of guava powder compared to plain guava extract. Overall the spray-dried Guava powder may be a good source of natural antioxidants and profoundly increase use of Guava in value added processing and dietary intake.

Keywords: *Psidium Gujava*; Guava extract; Antioxidants; Phenolic compounds; Antimicrobial activity; Spray-drying; HPLC

Introduction

Guava (*Psidium Gujava*) is a popular fruit of tropical and subtropical countries. It belongs to Myrtaceae family. The fruits are juicy and sweet in taste, with red or white flesh pulp and many seeds within. The fruit contains 80% moisture 20% dry matter, 1% ash, 0.7% fat and 1.5% protein [1]. Guava is rich source of ascorbic acid (vitamin C) and contains other nutraceutical components vitamin (A) beta-carotene, vitamin (B_1) thiamin, (B_2) riboflavin, niacin and pantothenic acid. In addition, it also contains a fair amount of phosphorous, calcium, iron, potassium and sodium [2]. The high level of antioxidants pigments like carotenoids and polyphenols present in guava increases its dietary value [3]. These various bioactive nutrients play significant role in traditional therapies for various health problems related to several diseases like diarrhea, diabetes (type 2) and obesity [4]. Although guava poses enormous health benefits still a major drive in the research and development of guava as important fruit is far behind the other exotic fruits [5]. The utility of guava as functional foods are increasing day by day with the main traditional use preferred as an anti-diarrhoeal agent for treatment of gastroenteritis, dysentery, stomache, antibacterial colic pathogenic germs of the intestine, other medicinal properties of guava includes treatment of diabetes, hypertension, inflammation etc [6]. The fresh fruit is preferred for consumption but seasonal availability limits consumption of fruit throughout the year. Hence processed products such as puree, paste, canned slices in syrup or nectar are developed and marketed. Guava juice is a health drink used by the population worldwide and manufacture of instant guava powder available for formulated drinks, baby foods and other confectionary products are gaining popularity. Typically, the production of a fruit powder requires heat to evaporate water from the fruit juices, and a grinding mechanism to convert the product to a powder form. Spray drying is a 1-step processing method for the production of powder because it eliminates the grinding step and limits the degradation of the nutraceutical components. The drier is a single-stage tower with a cyclone and a product receiver cyclone for the conveying system. The product is held in a balance tank, and passes through a high-pressure pump up to the spray nozzle. The air heating system is a direct gas-fired and the air is filtered before going into the heating chamber. The feed is preheated to approximately 60°C with constant stirring and then transferred to the balance tank of the spray dryer [7]. The present study aims to explore the physiochemical properties, phenolic and flavanoid constituents of spray dried guava powder with evaluation of its antioxidant capacity and antimicrobial activity on the causal agents of intestinal infections in humans.

Materials and Methods

Plant material collection and spray drying of guava fruit powder

The fresh Guava fruit of variety Allahabad *safeda* were collected from local market of Varanasi, India in February 2013. Fruits were selected at fully ripened stage, free from insect or any mechanical damage. The whole fruit was washed thoroughly and rinsed three times, cut into small pieces in a fruit mill and transferred to a water bath (Model: Gyromax-929, UK) and the temperature was maintained at 30°C with agitation. The pulp was further heated at 50°C for 30min. The enzyme inactivation was accomplished by heating the pulp to 90°C for 5min. The soluble extract was diluted in distilled water until reaching a total soluble solid content of 12°Brix, and blended in the laboratory blender (Phillips, India) for complete homogenization. The resulting guava soluble extract was kept at the temperature of 20 ± 1°C for 30 min and passed through the micro-filters (Applieo Membranes Inc., USA) of 100 mm mesh. The spray drying was performed according to

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Received July 21, 2014; **Accepted** August 26, 2014; **Published** September 03, 2014

Citation: Chauhan AKr, Singh S, Singh RP, Kumar A (2014) Determination of Antioxidant Capacity, Total Phenolics and Antimicrobial Properties of Spray-Dried Guava Extract for Value-Added Processing. J Food Process Technol 5: 368. doi:10.4172/2157-7110.1000368

the optimized protocol done in laboratory [8]. The spray-dryer used was a Lab Plant spray dryer with centrifugal atomizer driven by an air turbine at speeds up to 40,000 rpm (Jay Instruments & Systems Pvt. Ltd model LSD-48, Pune, India). Following spray drying parameters were taken, the feed was preheated to approximately 60°C with constant stirring and then transferred to the balance tank of the spray dryer the spray drying conditions were kept constant for each run with air inlet temperature at 170°C and air outlet temperatures of 85°C by regulating the feed pump speed. Maltodextrin concentration added was 7% w/v **(Risimaldex; DE 20,** Riddhi Siddhi Gluco Boils Ltd., Ahmedabad, India). Powder was collected from hot air by a cyclone and kept in PE-PA (60:40 w/w) laminates wrapped with aluminum foil and stored in desiccators containing silica gel. A total of (100 g) samples of spray dried guava powder and fresh guava fruit (grounded to soluble extract using a homogenizer) was dissolved in 100 ml distill water and filtered kept at 25°C until analysis.

Guava soluble extract prepared for analysis

Guava soluble extract (100 ml) were used for extracting fresh *P. guajava*. The first extraction was done for 3 h and the other was carried out for 16 h with hexane (2×600 ml). An orbital shaker was used to facilitate extraction. The extracted material was filtered, pooled as appropriate and concentrated en vacuo to give 2.4 g of Guava sample.

Proximate composition

The chemical composition of guava powder and guava soluble extract was determined according to standard methods [9]. Moisture content of samples was determined by drying at the temperature of 105°C in the oven until a constant weight was obtained. Ash was determined gravimetrically by incineration in a muffle furnace (Remi, New Delhi, India) for 24 h at 550°C. Crude protein content was determined using Kjeldahl method (Kel – plus, India). Lipids present in the product were estimated by extraction of a known weight of sample with petroleum ether in a rapid Soxhlet extraction apparatus (Soxtherm SE-416, Gerhardt, and Bonn, Germany). Carbohydrate content was calculated by difference. The pH value of powder sample was measured by blending 5 g powder with 25 mL deionized water at 20°C, using the electronic pH meter (Orion 2 star, Thermo scientific, USA) calibrated with standard buffers. The total soluble solids content for guava sample were analyzed using a RFM Refractometer (Model: ATAGO-28E, Erma Hand Refractometer, India,) equipped with a percentage sugar scale and expressed as °Brix. The titratable acidity was calculated using a standard 1% phenolphthalein solution, titrated against 0.1N NaOH and the result was expressed as grams of anhydrous citric acid per 100 g of sample. The samples were measured in triplicate for all the analysis performed.

Determination of Ascorbic acid, total carotenoids

Ascorbic acid content was determined according to [10]. Official Method 985.33 (2, 6-dichloroindophenol titrimetric method). Ascorbic acid content is expressed as mg/100 g DW. Total carotenoids were determined according to the procedure given by [11] as follows: 5 g of guava sample were extracted with a mixture of acetone and petroleum ether (1: 1, v/v) repeatedly until a colorless residue was obtained. The upper phase was collected and combined with crude extracts after being washed for several times with water. The extracts were made up to a 100 ml with petroleum ether. Total carotenoids content was determined by recording the absorbance at 451 nm with a spectrophotometer. Total carotenoids were estimated by mg/100 g DW. The samples were measured in triplicate for all the analysis performed.

Determination of total polyphenols, total flavanoids

The amount of total phenolics was estimated using Folin–Ciocalteau reagent [12], Each sample (10g) taken was diluted with distill water at five different concentration (20%, 40%, 60%, 80% and 100%) upto 10 ml and then homogenized by adding 80% aqueous ethanol, at room temperature and centrifuged (Eppendorf C-5702, Japan) in cold at 10000 rpm for 15 min at 4°C, and the supernatant was collected and evaporated to dryness at room temperature. Residue was dissolved in 5 mL of distilled water. 100 µl of this extract was diluted to 3mL with water and 0.5 mL of Folin-Ciocalteu reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was added, and the contents were mixed thoroughly. After standing for 60 min at room temperature, the absorbance was measured at 650 nm. Phenolic contents were calculated on the basis of the standard curve for gallic acid (GAL). The results were expressed as mg of gallic acid equivalent per 100 g DW. The flavonoids were estimated as [13]. A total of 25 mg of sample was dissolved in distill water and evaporated to dryness, to this 4 ml of vanillin reagent was added and heated for 15 min in boiling water bath. The absorbance was read at 340 nm. The values are expressed as mg flavonoids/g.

Measurement of antioxidant activity by DPPH assay

The DPPH assay was based on the procedures described in [14]. Fresh and spray dried powder sample were taken in five different concentration (20%, 40%, 60%, 80% and 100%) and diluted with 100 ml distill water, then samples was extracted by homogenizing 10 ml sample with 100 ml methanol for 1 min and centrifuged (Eppendorf C-5702, Japan) at 10000 rpm for 15 min at 4°C. The clear supernatant was transferred to a glass bottle and measured immediately used for total antioxidant activity, 1g of fruit sample was dissolved in 0.5 ml methanol and 0.15 mM DPPH solution was added to it. The contents were mixed vigorously and allowed to stand at 20°C for 30 min in dark. Reading was taken at 517 nm. The antioxidant capacity of each sample was estimated in triplicate, at different concentration. The free radical scavenging activity was expressed as ascorbic acid (AA) equivalent antioxidant capacity, in mg AA/100 g using the equation: FRS=IC50(AA)/ IC50(sample)×105. IC50 of AA used for calculation of FRS was 0.00387 mg/mL.

Analysis of phenolic compounds using HPLC

Phenolic compounds of methanolic extract of raw and processed Guava were identified using a method introduced by [15]. Briefly HPLC analyses was performed on HPLC–1100 system, equipped with a thermostatically controlled column oven and a UV detector set at 280 nm (Hewlett-Packard, Palo Alto, CA, USA). Samples and mobile phases were filtrated through a 0.45 mm Millipore filter, type GV (Millipore, Bedford, MA, USA), prior to HPLC injection. Each sample was analyzed in triplicate. The identified phenolic compounds were quantified on the basis of their peak area and compared with calibration curves obtained with the corresponding standards and then expressed as mg/100 g of extract.

Statistical Analysis

All the assays were performed in triplicate and the data from the dried powders were compared to fresh soluble extract of guava juice. Data were statistically analyzed in completely randomized design in factorial arrangement, according to the procedures outlined by [16], and the treatments means were compared by least significant differences (LSD) and Duncan' multiple range using SPSS program package.

Determination of antimicrobial activity

Test microorganism: The extracts were tested against common pathogenic microorganisms, including *Escherichia coli*, *Shigella*, *Klebsiella*, *S Typhi*, *Psuedomonas aerugionsa*, *Candida popecelis, C. bulboni and C albicans.* Microorganisms were maintained and stored frozen in bead vials by Microbiology Department, Institute of Medical Sciences, and B.H.U).

Determination of antimicrobial activity: The experiment was conducted according to the disc diffusion method [17]. The sample extract was prepared by dissolving 5 g of guava powder in 100 ml of distill water, mixture was agitated for 12 hrs in room wrist action shaker and filtered with Whatman paper number 1, for stock solution. A standard antibacterial agent, norfloxacin (Belco Pharmaceuticals, Varanasi) and an antifungal agent, fluconazole served as a positive control. Dimethylsulfoxide (HPLC grade) was used as a negative control. Bacterial strains were revived in 10 mL of nutrient broth (HiMedia Laboratories Pvt. Ltd., Bombay, India) to attain a final density of 10^7 CFU per ml. The cultures were revived and diluted 10-fold in Ringer's solution (LabM) for the inoculation of 10 ml of nutrient broth incubated at 37°C overnight to give an initial suspension of 10-100 cfu/ml. The sterile filter paper Whatman no. 3 (6-mm diameter) was cut into a disc form of and placed in the inoculated agar plates containing indicator microorganisms, 5 µl aqueous extract of Guava powder was added to the disc and incubated at 37°C for 24 h for microbial growth. After incubation, the inhibition zones were estimated by taking photos of plates and processed using Impulse Vision XL 2.5 software. Each inhibition zone diameter was measured three times and the average was taken [18].

Results and Discussion

Physiochemical properties

The chemical composition analysis of spray dried and soluble guava extract are presented in Table 1. The samples showed mean pH of 3.85 ± 0.21 standard deviation, SD), moisture content of 54. 2 ± 2. 6 per 100 g in parentheses with up to 90% water content in fresh guava rather than 70% water content in spray dried guava powder. The protein, fat and carbohydrate content was 5.0 ± 0.21 SD, 4.0 ± 0.21 SD and 38.6 ± 0.6 SD. The total ash was (0.7 and 0.5) for fresh and spray dried guava powder respectively [19]. Ascorbic acid is one of the most sensitive vitamins. For this reason, it is often used to evaluate the influences of food processing on vitamin contents. Results show increase in Ascorbic acid content of spray dried guava powder over fresh soluble extract this must be due to increase in the concentration of guava concentration at constant temperature and pressure while spray drying. Boiling leads to maximize ascorbic acid losses however pressure steaming causes more retention of the losses [20]. Results show that on basis retention of pigments between soluble extract and spray dried powder no significant difference was observed in total carotenoids content (Figure 1). Carotenoids compounds are group of aliphatic-alicyclic, fat soluble pigments, content depends upon the yellow-to-red compounds responsible for red, orange, and yellow color of edible fruits and vegetables and widely distributed in nature [20]. Phenolic compounds (PCs) may contribute directly to the antioxidant action; therefore, it is necessary to investigate total phenolic content. Phenolic compounds in fruits are present in both soluble forms and combined with cell-wall complexes [21]. The TPC values in this experiment are in good agreement with previously published values of tropical fruits stating that the experiments conducted were fairly conducted [22]. The total

Composition (%)	Guava soluble extract	Guava powder
Moisture	86.9	81.3
Protein	5.1	4.9
Fat	4.8[a]	3.2[b]
Ash	0.7	0.5
Titratable Acidity (%) as citric acid	1.27[c]	1.34[d]
pH	3.1	4.5
TSS (° Brix)	12.5[a]	15.[1b]
Brix/Acid ratio	9.84	13.50
Ascorbic Acid (mg/100g)	98.2[c]	118.6[d]
Pectic substances (%)	11.9	5.52
Total sugars (%)	9.94	7.61

Table 1: Proximate composition of guava soluble extract and spray dried powder (a & b, c & d Significance (P>0.5)).

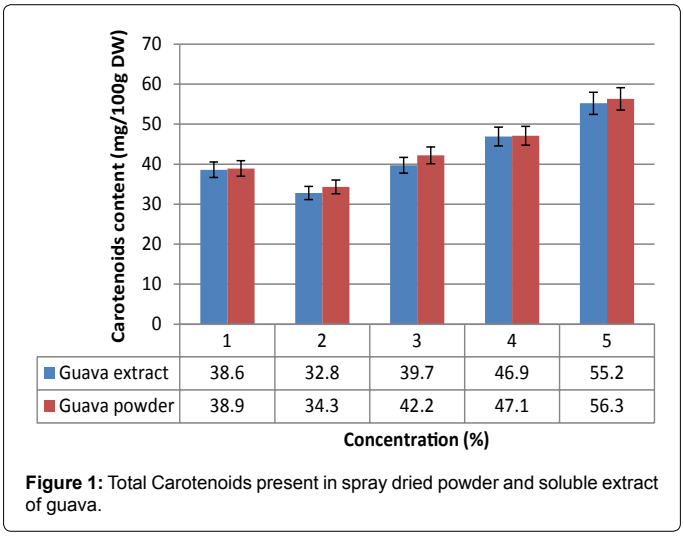

Figure 1: Total Carotenoids present in spray dried powder and soluble extract of guava.

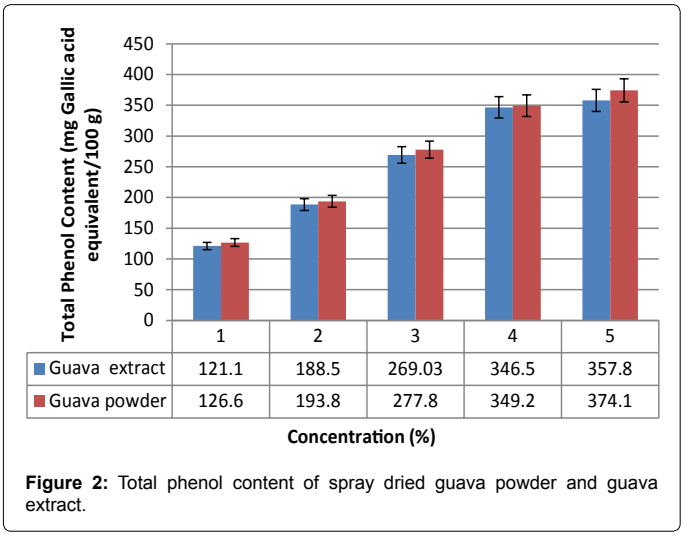

Figure 2: Total phenol content of spray dried guava powder and guava extract.

phenol content (TPC) of spray dried guava powder when compared with guava soluble extract was found significant (Figure 2), this is due to the high concentration of guava powder after spray drying and the protective coating of maltodextrin which gave maximum retention of the total phenolic content in guava powder [7]. Results are presented per gram of soluble extract or spray-dried material, to demonstrate the concentration effects. Spray dried guava powder showed fair amount

of total phenolics, when compared with soluble extract at five different concentrations. The total polyphenolic content for red strawberry guava as reported earlier was similar to this study [22]. The total flavonoid content decreased due to spray drying in guava powder (Figure 3), this must be due heat sensitivity of the pigment, during processing of fruits and vegetables boiling or heating at high temperature even for 5min causes reduction in total flavonoids content of the product. However the process of slow steaming or microwaving of fruits and vegetables had proved least losses of the pigment in previous experiments [23]. While processing the fruits, into powder form for the purpose of value addition, this factor should be given importance to reduce the loss of flavonoids as it has wide range of biological activities such as cell proliferation-inhibiting, apoptosis-inducing, enzyme-inhibiting, antibacterial, and antioxidant effects [24]. The spray dried guava powder showed 264.3 mg/100 g of polyphenolic content, previous study on comparative analysis of antioxidant activity and composition of red and yellow strawberry guava fruits also reported (501.33 mg/100g and 292 mg/100 g) of polyphenolic content, this values are in close agreement, and processed guava fruit powder in our study is beneficial to be used as function food product. The reason of better retention of total phenol content during spray drying process at high temperature is encapsulation with carrier agent maltodextrin, since long duration of cooking at high temperatures had been observed as the main causes of loss of nutrients which causes disruption of cell wall and breakdown of biochemical compounds, also the 1 step processing of spray drying reduces the time of contact of high temperature with sample and reduces losses of essential components [25]. Other reports also show total phenol content of 100 to 320 mg/100 g on a fresh weight basis for blackberry, blueberry, and strawberry before and after processing [26].

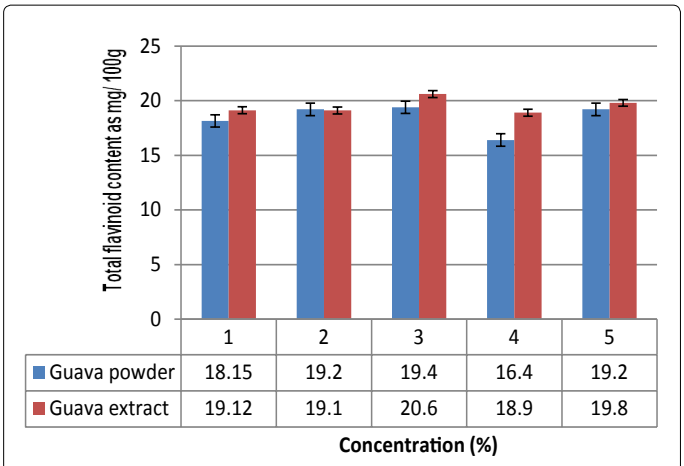

Figure 3: Total flavonoids content of spray dried guava powder and guava extract.

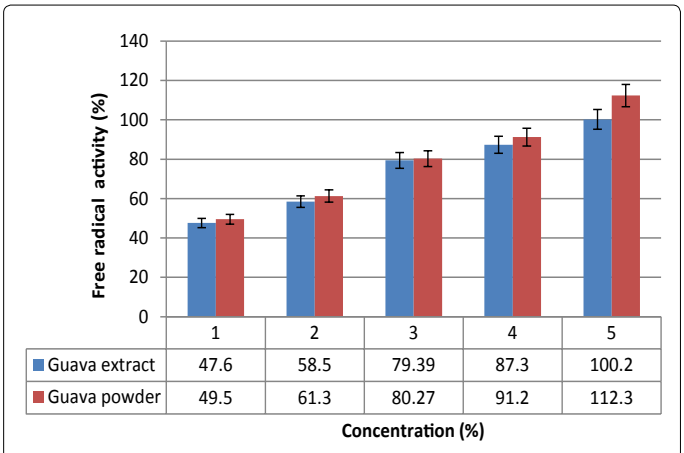

Figure 4: DPPH Activity (%) of spray dried guava powder and guava extract .

Antioxidant activity

Comparison made between both samples for DPPH activity showed significant variations (Figure 4). Here the antioxidant activity of spray dried guava powder consistently ranked high when compared to guava soluble extract at five different concentrations. Other reports on the antioxidant capacity of ethanolic extract of *P. Guajava* in terms of FRAP activity has been reported high [27]. These results supported the increased potential of processed guava to be promoted for value addition. The dried guava powder showed good retention of antioxidants when compared with soluble extract through DPPH activity, which confirms that spray drying process does not affect the antioxidant capacity of the fruit powder. Earlier studies on the antioxidant activity of Guava through different extraction procedures found aqueous extraction through simple aqueous homogenization was best procedure [28]. This comparative study between guava powder and guava soluble extract showed higher quality of guava powder was maintained after processing. Other studies on fruits like blueberry, aril and peanut had also showed comparison between processed and soluble extract of fruits characterized by higher concentration effect due to removal of other insoluble factors [29].

Test organisms	Spray dried Guava Powder	
	Zone of Inhibition (cm)	MIC*(mg/ml)
Escherichia coli,	2.0 ± 0.3	8 ± 0.1
Shigella,	3.2 ± 0.2	11 ± 0.4
Vibrio cholera	NF	-
Klebsiella,	1.0 ± 0.1	5 ± 0.3
Salmonella typhi	NF	-
Psuedomonas aerogionsa	NF	-
Candida popecelis	2.7 ± 0.2	0.2 ± 0.01
Candida bulboni	6.0 ± 0.3	0.4 ± 0.1
Candida albicans	5.2 ± 0.5	0.7 ± 0.2

Table 2: Antimicrobial activity of spray dried guava powder (* Minimum Inhibitory Concentration (mg/ml)).

Bioactive compounds characterized by HPLC

Presence of qualitative phytochemicals was further quantified by HPLC analysis of the two samples (Table 2). The results revealed the presence of quercitin as major component while caffeic acid and p-coumaric acid were also found in high proportions (Figure 1). Guava has been established in the literature as a particularly rich source of polyphenols [30]. HPLC analysis was used to get more precise information of individual compounds over total phenolic content. The contents of eight types of polyphenolic compounds were identified namely Vanillic acid, Syringic acid, Quercetin, Ferulic acid, Cinniamic acid, Gallic acid, Courmic acid and Salicylic acid in Guava extract and spray dried powder (Table 3). These compounds have been identified according to their retention time and the spectral characteristics of their peaks compared to those of standards as well as by spiking the sample with standards. The quantities of the identified compounds are expressed in mg/100 g DW. The predominant phenolics of Guava were gallic acid (58.9), quercetin (53.95), pyrogallol (18.9), vanillic acid (15.79), coumaric acid (6.94), and kaempferol (25.91) mg/100 g

DW, respectively (Table 1). Certain unresolved peaks were also seen in spray dried sample that may be polymeric in nature; this may be due to addition of maldodextrin while processing Guava powder. The significant levels of phenolic components in spray dried sample clearly indicate that excluding polymers there was no significant difference among the total concentration of phenols between guava extract and spray dried powder. Bioactive compounds in guava extract were sensitive to heat and can be easily oxidized when subjected to high temperature, during spray drying to avoid this factor an optimum yet constant temperature and vacuum conditions were maintained which retained the losses in bioactive compounds [31]. The breakage in molecules due to spray drying resulted in redistribution of molecular weight, but overall quantities were retained. Such redistribution may benefit the in vivo absorption of total phenols present in Guava which is primarily dependent on the size of the molecule (Figure 5). Monomeric and dimers molecules are easily absorbed than larger molecules and pass into the colon, where they are fermented by gut microflora. Therefore, spray-drying of guava extracts has the potential to enhance polyphenolic bioavailability in vivo. It is worthy to mention that polyphenol losses could also be due to the covalent binding between oxidized phenols and proteins or amino acids as well as the polymerization of oxidized phenols [32].

Antimicrobial properties

The antimicrobial activity was measured by disc diffusion assay for guava samples against nine test organisms. The aqueous extract of fresh

Name of standard	Spray dried Guava			Fresh Guava		
	R.T.	Area	conc.	R.T.	Area	conc.
Vanillic acid	14.932	3534976	15.709218394	14.675	403639	3.680350305
Syringic acid	15.943	4529087	-	14.823	260199	-
Querctin	33.31	1874373	53.95900389	33.269	1011393	4.604747049
Ferulic acid	22.616	9656083	-	22.252	208553	-
Cinnamic acid	27.008	4580399	7.358736502	25.003	245602	6.358737803
Gallic acid	7.093	16639408	58.98798623	24.184	1037018	15.36469927
Coumaric acid	20.149	26093796	-	20.856	371808	3.562226056
Salicylic acid	24.922	4395039	31.3321564	24.217	482437	27.44213419

Table 3: HPLC analysis of spray dried and fresh Guava extract. (RT is Retention Time, Conc. concentration (ppm, part per million)).

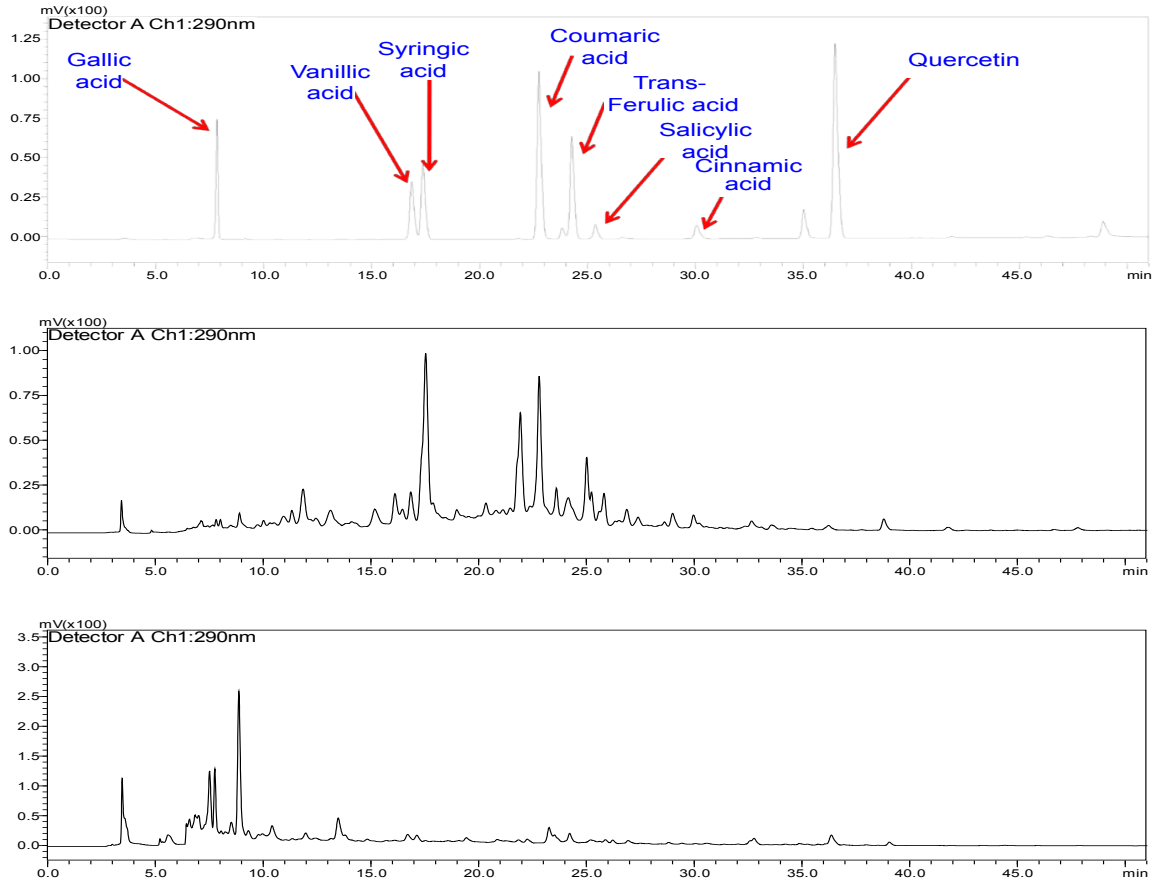

Figure 5: HPLC chromatograms of the standard, fresh and spray dried Guava extracts.

guava extract showed maximum activity against both gram positive and gram negative bacteria while aqueous extract of spray dried guava showed higher activity against gram positive bacteria but least activity against gram negative bacteria (Figure 6). The zone of inhibition was highest for *Listeria monocytoges, S. aurens* and *Shigella sp., Psuedomonas aeroginosa*. For the remaining test organisms, zones were relatively small and ranged from 8.3 to 13.3 mm. None of the guava extracts showed activity against *S. Typhii*. The aqueous extracts of both samples was significantly effective on the growth of fungal microorganism of *Candida* species, *popecelis, bulboni* and *albicans* from which *C. bulboni* showed maximum inhibition (6mm). In the previous investigations, it was reported that *guava* leaves and bark extract were tested for their antimicrobial activity using the agar diffusion technique, against bacteria such as *S. aureus, E. coli, P. aeruginosa*, and the fungus *C. albicans,* while our results showed that the aqueous extract of guava fruit powder can inhibit the growth of *S. aureus, E. coli, P. aeruginosa*, and the fungus *C. albicans* by the disc diffusion method [33]. The presence of significant amount of carbohydrates, phytochemical and flavonoids in spray dried guava powder indicated the presence of glycosides, steroids, and tannins which may be responsible for antimicrobial activity of the test extract. Since the flavonoids are hydroxylated phenolic substances in C_3–C_6 unit linked to an aromatic ring are synthesized by plants in response to microbial infection [34-40]. Their activity is probably due to their ability to complex with extracellular and soluble proteins, and to complex with bacterial cell walls. More lipophilic flavonoids may disrupt the microbial membranes. This could be beneficial for developing formulations from natural produce for therapeutic applications.

Conclusion

The results showed that the spray dried guava powder was similar in physiochemical properties to fresh guava. The spray dried guava powder showed good antioxidant capacity and antimicrobial properties to inhibit all of the bacteria and fungi used in this study with different degree of inhibition. Hence, the spray dried guava powder is effective against the tested bacterial and fungal strains. In conclusion, spray-drying is potentially a useful process for large scale production of dried powders containing natural products which may act as alternative for antibiotics and chemotherapeutic agents in disease management. However this needs to be investigated if the original characteristics are maintained in every possible aspect after processing. Furthermore, the numerous applications of guava powder is not limited to commercial products like jam, jellies etc, but can be used as health supplements, it is possible that the resulting powder could be used in a wide range of functional food applications, delivering antimicrobial properties in those foods.

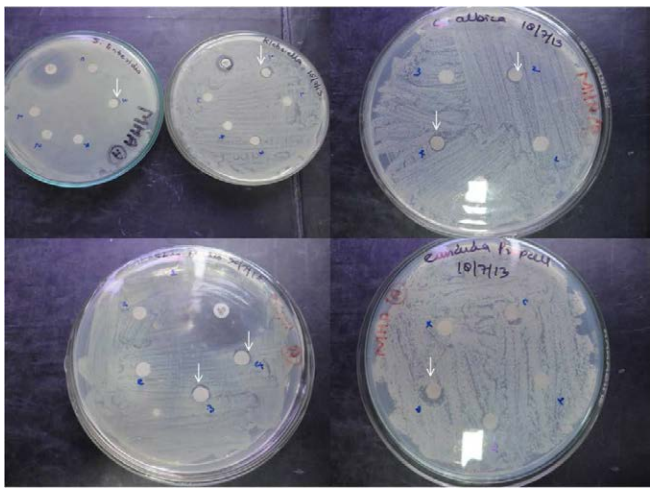

Figure 6: Inhibitory effect of spray dried Guava powder on test microorganisms.

Acknowledgement

The authors gratefully acknowledge funding from Ministry of Food Processing Industries and Banaras Hindu University, for the undertaking of this project. There authors also declare no conflict of interest.

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