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Chemical characterisation of pulp, seed powder and a ready-to-drink juice produced from *Syzygium cumini* fruit

M. Wasswa[1], G.A. Tumuhimbise[1*] and H. Acham[1]

[1]Department of Food Technology and Nutrition, School of Food Technology, Nutrition and Bioengineering, College of Agricultural and Environmental Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda

*Corresponding author: ampston23@gmail.com

Abstract

Despite the nutritional potential of *Syzygium cumini,* commonly known as black plum, there has been limited utilisation of the fruits (pulp and seeds) and yet it has capacity to significantly contribute to food and nutrition security. The purpose of this study was to explore the possibility of utilizing *S. cumini* fruits in the production of a nutritious and acceptable juice. Ripe *S. cumini* fruits were randomly sampled from Nakasero Market, Kampla. The fruits were bulked and processed to obtain pulp and seeds. The seeds were separated from pulp, dried and milled into powder while the pulp was processed into juice. The seed powder was used in the formulation of the ready-to-drink juice at 0, 1.25 and 5%. The sensory evaluation was carried out to ascertain the acceptability of juice in which the seeds of *S. cumini* have been added. The pulp contained total phenolic concentrations in the order of 27.7±2.06 mg 100 g^{-1}; while dried seed powder and juice contained 57.2±4 and 10.6±1.25 mg ml^{-1}, respectively. The levels of flavonoids were less in juice (5.7±1.0 mg 100 $^{-1}$ g) compared to pulp (36.00±5.95) and seeds (457.2±31.44 mg 100 $^{-1}$ g). The total antioxidant activity (TAA) in terms of 1,1-diphenyl-2- picrylhydrazyl (DHHP) percentage inhibition for pulp, seed powder and ready to drink juice were 85.75±0.54, 93.94±0.43 and 25.30±6.07%, respectively. Sensory acceptance reduced with increased levels of dried seed powder incorporated into the juice. On the other hand, the amounts of antioxidants increased in the different juice samples with increase in amounts of dried seed powder.

Key words: Anti-oxidants, black plum, flavonoids, phytochemicals

Introduction

Black plum (*Syzygium cumini)* is a fruit tree of Indian origin (Swami *et al*., 2012). It grows widely in different agro-climatic conditions in many parts of Asia, Africa and South America (Aqil *et al.,* 2012; Patel and Rao, 2014). The fruit has a taste varying from acidic to fairly sweet, with astringent properties. Nutritional and medicinal properties of the leaves, seed and bark of the black plum have been documented (Sehwag and Das, 2015).

The fruits are commonly consumed in their natural form, but can be used to produce different products such as juice, wine, preserves, jellies, jams and vinegar (Kannan and Puraikalan, 2015), or dried into powder which can be added to different foods for enrichment (Sehwag and Das, 2015). *Syzygium cumini* seeds contain alkaloid and glycosides which are thought to reduce or stop diastatic conversion of starch into sugars. Seeds of *S. cumini* are antiscorbutic, diuretic, and, therefore, affect urinary discharges, which makes it important in the treatment of diabetes (de Carvalho Tavares *et al*., 2016), diarrhoea and dysentery (Patel and Roa, 2014). Thus, products like seed powder and volatile oil extract from black plum seeds and leaf extracts may be used as an effective remedy for diabetes (Bhowmik *et al*., 2013), heart as well as liver disorders (Faria *et al*., 2011). Generally, *S. cumini* contain polyphenols that are a large group of phytochemical and research shows that these are becoming popularly accepted due to health benefits associated with them and foods in which they are contained (Nisha *et al*., 2009).

Despite all these important uses of black plum, it is one of the most neglected fruit tree species in many parts of the world. The food industry has hardly utilised this plant in producing value added products. In some areas, this tree species faces extinction because they are indiscriminately cleared for settlement, agriculture and for fuel. Celli and Pareira-Netto (2011) noted that characterisation of the antioxidant properties of this fruit is important for the food industry, especially in the manufacture of new health oriented products. In order to stimulate new ways of using this fruit tree, this study was designed to characterise the black plum pulp and seed powder and then use them in the production of nutritious and acceptable ready-to-drink juice.

Methodology

Materials

Fully ripened black plum fruits were obtained from Nakasero Market in Kampala City in Uganda. Fleshly harvested clean fruits were randomly selected from vendors, packed into polythene bags and placed in cold boxes. The fruits were then transported to the research laboratories at the School of Food Technology Nutrition and Bioengineering, Makerere University. They were washed using portable water and kept at refrigeration temperatures awaiting use in the study. Chemical reagents for laboratory analysis were obtained from BDH Supplies, Kampala, Uganda.

Preparation of the pulp and juice

Whole refrigerated *S. cumini* fruits were allowed to thaw at room temperature (24 ^{o}C) for about an hour, and later washed using portable water. Approximately 7 kg of cleaned fruits were manually separated into flesh/skin and seed; and the former blended in a warring blender (Lila ram Manomal and Sons, India) into pulp.

To make the juice, the resultant pulp was sieved using a cheese cloth to produce a concentrated juice, to which water was added in a ratio of 40:60 (*S. cumini* concentrated juice:water). Table sugar (sucrose) was then added until 14.5^{o} Brix was achieved. The soluble sugar content in ^{0}Brix was measured using a refractometer (Portable Refractometer, Mettler, Toledo, UK). The mixture was pasteurised at 85 ^{o}C for 15 seconds during which 50 ppm of sodium benzoate, sodium sorbate and citric acid were added in order to preserve the juice. The juice (13.5 L) was then manually hot filled into 3 different clean sanitised 5 L containers.

Preparation of black plum seed powder

Seeds were separated from the whole fruit, washed with portable water and dried under shade for about 4 days, and then crushed to fine powder using a grinder (Lila ram Manomal and Sons, India). The seed powder obtained was used in formulation of the black plum juice.

Laboratory procedures

Moisture content of black plum seed powder and juice was measured following a method described by AOAC (2000). This was achieved by taking 5 g of the pulp concentrate and seed powder separately into aluminium containers of known weight and then heated in a dry air oven at 105 ^{o}C for about 24 hr. Moisture content was then computed gravimetrically.

Ash content

Determination of ash content, followed AOAC (2000) method; whereby about 10 g of each sample was taken in a silica crucible. The crucible was heated in a muffle furnace for about 3-6 hr at 600 ^{o}C. It was cooled in a desiccator and weighed after completion of ashing (ash became greyish white). The weight of ash was also computed gravimetrically using the following formula.

$$\text{Ash\%} = \frac{\text{Weight of ashed sample}}{\text{Weight of sample taken}} \times 100$$

Crude fat

About 3-4 g of pulp or seed powder was taken into a thimble lined with a circle filter paper. The thimble and contents were placed in 50 mL beaker and oven-dried for 6 hr in at 100-102 ^{o}C. The extraction flask was weighed and approximately 85% of petroleum ether added. The sample contained in the thimble was extracted with petroleum ether for 3-4 hr in a Soxhlet extraction apparatus. Upon completion of the extraction, the Soxhlet unit was taken apart and the flask and its contents placed in a convection oven and heated at 102 ^{o}C for a time required to obtain constant weight (at least 4 hr). The flask and contents were cooled in a dessicator and weight of the flask and extracted oils weighed (AOAC, 2000).

Where:

W_1 = weight of sample (g), W_2 = weight of flask + sample after extraction, and W_3 = weight of flask only (g).

Crude protein, dietary fiber and carbohydrates

The crude protein was determined using micro Kjeldahl method as described in AOAC (2000); while dietary fibre was determined using non enzymatic gravimetric method as described by AOAC (2000). The fibre content was calculated as follows:

$$\%dietary\ fibre = \frac{W2-W1}{W1}\ X100$$

Where:

W_1 = weight of sample before, W_2 = weight of fibre. Carbohydrate in the sample was calculated by difference method as described by Shahnawaz *et al*. (2009) as follows:

% carbohydrates = Total solids - (% ash-% fat-% protein).

Analysis of phyto-chemicals

Determination of phytochemicals such as total flavonoids, total phenolics and total antoxidant capacity were determined using a method described by Nankunda *et al*. (2015). Total carotenoids in the pulp, seed powder and a ready to drink juice (plain juice) were determined according to the method described by Rodriguez- Kimura and Amaya (2004); while ascorbic acid, total sugars and reducing sugars were determined using AOAC (2000) methods.

Sensory evaluation of black plum juice

Sensory analysis of the black plum juice involved the participation of a consumer panel of 40 trained panelists, who had been adequately trained in carrying out sensory tests. The panellists comprised of 23 women and 17 men, mainly staff and students from the Department of Food Technology and Nutrition at Makerere University. Each panellist evaluated six sensory characteristics, namely; overall acceptance, taste, colour, flavour, after taste and thickness of the enriched juices and control. Each sensory attribute was rated on a 9-point Hedonic scale. The rating scale ranged from 9 = like extremely, to 1= dislike extremely (Carr *et al.*, 1999). Each panelist was given three samples with three digit codes. A control juice sample (without black plum seed powder) and 2 samples with different black plum seed powder formulations (1.25 and 5% amounts of seed powder w/v) were served to panellists. The panelists were presented with 100 mL samples of each juice at room temperature, under normal lighting conditions. The black plum juice and control samples were prepared the day before the sensory experiment and stored under refrigeration conditions. Bottled water was provided to rinse the mouth between tasting different samples.

Statistical analysis

Data were obtained in triplicate and analysed using SPSS version 20.0 (IBM SPSS Statistics for Windows (version 20; IBM Corporation, Armonk, New York). Means and standard deviations were derived for the different samples for all attributes of the proximate composition, sensory acceptability, phytochemicals and functional properties of the black plum juice that was produced. The effect of different treatments was determined using ANOVA at an alpha value of 0.05 and the means were separated using Tukey's test.

Results

Chemical characteristics

The moisture content, ash content, dietary fibre, crude fat, crude protein and carbohydrates (nitrogen free extract, NFE) for the pulp and the seed powder are shown in Table 1. The results showed that there was a significant difference ($P < 0.05$) for all proximate parameters of pulp and seed powders, except crude protein. The levels of various anti-oxidant substances in the black plum are shown in Table 2. Generally, the results show that there was a significantly ($P < 0.05$) higher level of antoxidant substances in seed powder, compared to that in pulp and juice. However, Vitamin C was highest in the pulp followed by juice but was lowest in seed powder.

Table 1. Proximate composition of the pulp extract and dried seed powder on dry matter basis (%) of black plum fruits obtained from Nakasero Market in Kampala, Uganda

Parameter sample	Moisture content (%)	Ash content (%)	Dietary fibre (%)	Crude fat	Crude protein (%)	Carbo-hydrates (%)
Pulp	83.3±1.15[a]	2.03±0.53[a]	2.08±0.60[a]	0.53±0.53[a]	1.81±0.1[a]	10.02[a]
Seed powder	11.77±0.16[b]	2.63±1.38[b]	3.92±0.38[b]	0.91±0.21[b]	1.93±0.2[a]	78.84[b]

Data are means of triplicate determination ± standard deviation. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$). Means were separated using Tukey's test

Table 2. Antioxidants analysis of the pulp extract and dried seed powder on dry matter basis (%) of black plum fruits obtained from Nakasero Market in Kampala, Uganda

Sample	Total phenolics (mg 100 g^{-1})	Total flavonoid (mg 100 g^{-1})	TAA (percentage DHHP Inhibition)	Carotenoids (µg 100 g^{-1})	Vitamin C mg 100 g^{-1}
Pulp	27.7±2.06[a]	36.0±5.95[a]	85.75±0.54[a]	0.60±0.00[a]	17.7±0.35[a]
Seed powder	57.2±4.57[b]	457.2±31.44[b]	93.94±0.43[a]	4.4±0.40[b]	0.33±0.06[b]
Juice (ready to drink (no additives)	10.6±1.25[c]	5.7±1.00[c]	25.30±6.07[b]	0.10±0.00[a]	9.2±0.35[c]

Data are means of triplicate determination ± standard deviation. Mean values in the same column with different superscript letters are significantly different (Tukey's test $P < 0.05$)

The results further indicate that samples in which dried seed powder was added contained significantly ($P < 0.05$) more antioxidants (total phenolic compounds, flavonoids and carotenoids) compared to samples in which seed powder had not been added (Table 3)**.** However, there was no significant ($P > 0.05$) change in vitamin C content of the juice in which the black plum powder had been added.

Sugar composition

The results showed that black plum seed powder contained more total sugars and reducing sugars than pulp (Table 4). However, the black plum pulp contained significantly ($P < 0.05$) more non-reducing sugars than in the seed powder.

Sensory evaluation

The sensory evaluation mean scores generally showed that the control sample (no seed powder added) was significantly ($P < 0.05$) more acceptable to panelists than samples where seed powder had been added (Table 5). Sample 425 (control) had

Table 3. Antioxidant levels of the three black plum juices enriched with dried seed powder

Juice treatments	Total phenolics (mg/100g)	Total flavonoid (mg/100g)	Carotenoids (µg/100g)	Vitamin C mg/100g
425 (Control; no seed powder)	10.6^{a}	5.7^{a}	0.10^{a}	9.20^{a}
364 (1.25 g of powder added to 100 mL of plain juice i.e. 1.25%)	11.32^{b}	11.42^{b}	0.16^{b}	9.20^{a}
837 (5 g of powder added to 100 mL of plain juice i.e. 5%)	13.46^{c}	28.56^{c}	0.32^{c}	9.22^{b}

Mean values in the same column with different superscript letters are significantly different ($P < 0.05$). Means were separated using Tukey's test

Table 4. Sugar composition of the pulp extract, seed powder of black plum obtained from Nakasero Market in Kampala, Uganda

Treatments	Total sugars (g/g)	Reducing sugars (g/g)	Non reducing sugars (g/g)
Pulp	0.132 ± 0.06^{a}	0.043 ± 0.04^{a}	0.087 ± 0.05^{a}
Seed powder	2.51 ± 0.06^{b}	1.35 ± 0.13^{b}	0.027 ± 0.03^{b}

Data are means of triplicate determination ± standard deviation. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$)

Table 5. Mean scores for sensory acceptance of juices from black plum obtained from Nakasero market in Kampala, Uganda

Treatments	Colour	Taste	Flavour	Thickness	Aftertaste	Overall acceptance
425	6.10 ± 1.67^{a}	6.93 ± 1.14^{a}	6.68 ± 1.53^{a}	5.83 ± 1.99^{a}	6.68 ± 1.51^{a}	6.73 ± 1.38^{a}
834	6.95 ± 1.47^{b}	6.63 ± 1.46^{a}	6.23 ± 1.91^{a}	6.35 ± 1.63^{a}	5.88 ± 1.70^{a}	6.60 ± 1.61^{a}
364	7.35 ± 1.69^{b}	4.68 ± 2.14^{b}	5.23 ± 1.99^{b}	6.12 ± 1.83^{a}	4.02 ± 2.17^{b}	4.80 ± 2.15^{b}

Data are means of triplicate determination ± standard deviation. Mean values in the same column with different superscript letters are significantly different ($P < 0.05$)

the highest scores, followed by 834 (5%) and then 364 (2.5%); that is 6.73±1.36, 6.60±1.61 and 4.80±2.15, respectively.

Discussion

Characteristics of pulp and seed juices
The results showed that pulp and seed powder values were significantly different for all parameters, except for protein and carbohydrates. The *S. cumini* proximate composition results obtained generally agree with those reported by Sartaji *et al.* (2015) affirming that the fruit is a good source of fibre and minerals. The required dietary allowance (RDA) for dietary fibre as stated by National Research Council of 1989 is 12 g per day. *Syzygium cumini* is a reliable source of fibre since on dry basis, 100 g seed powder are adequate to provide the RDA for dietary fibre.

Proximate composition on dry basis shows that the ash content, fibre content and carbohydrate (nitrogen free extract) are higher in seed powder than in pulp. Ash is the inorganic component after the removal of the organic component and water. There are variations in the proximate composition values of the fruit pulp and dried seed powder and this is mainly attributed to the natural occurrence of various substances in different parts of the plant. All in all, the results show that the formulated juice contained adequate nutrients that can significantly contribute towards the nutritional requirements of individuals.

Antioxidant analysis
The results show that the seed contains the highest level of antioxidant (except for vitamin C) and TAA (total antioxidant activity). The results further indicate that there is a considerable level of antioxidants in the various parts of the black plum analysed. This is important since much focus is now put on fruits and vegetables that contain antioxidants since, they are considered to be of nutritional and medicinal value.

These results are important in as far as utilisation of the black plum is concerned. As remarked by Celli and Pareira-Netto (2011), making characterisation of the antioxidant properties of this fruit is a cornerstone in the food industry, especially in the manufacture of new health oriented products.

Total phenolic content
The different components contained different levels of total phenolics in the order juice <pulp <seed powder. In a similar research about the fruit Benherlal and Arumughan (2007) found that the levels of total phenolics in pulp and seed were 390 and 900 mg 100 g^{-1}, respectively. Although these values are higher than what was

obtained in the present study, perhaps as a result of differences in variety and sample extraction methods, nevertheless this confirms the high levels of phenolics in the fruit, especially in the seed.

From the research carried out by Grosso *et al.* (2014), the mean intake of polyphenols was recorded to be 1756.5 mg per day. Based on the present study, this amount can be obtained from intake of 7 g of pulp, 4 g of seed powder (which could be added in tea as the vehicle food) or 1.5 L of the juice. Other fruits, vegetables and beverages such as tea, coffee and wines are also considerably good sources of phenolic compounds and other antioxidants at large in our diets (Hertog *et al*., 1993). Different kinds of tea (black and green tea) contain between 61 to 250 mg 100 g^{-1} of phenolics on dry weight basis as noted by Balasundrum *et al*. (2006).

Total flavonoids

The total flavonoid levels in seed powder were significantly more than the levels in pulp and juice. Benherlal and Arumughan (2007) found out that the levels of total phenolics in pulp and seed were 4.0 and 80 mg 100 g^{-1}, respectively. The results in this experiment agree with the findings in the present study stating that seeds contain higher levels of flavonoids than the pulp.

Flavonoids are known to be the most sundry and widespread group of natural compounds, and are thought to be the imperative natural phenolics. Like other antioxidants flavonoids, have ability to scavenge free radicals (Ghafar *et al.,* 2010). It is difficult to report the recommended amount of antioxidants required with respect to the body needs (recommended dietary allowances, RDA). According to Chun *et al.* (2007), the estimated daily intake of flavonoids range from 150 to 215 mg d^{-1} across a range of age, gender variations and this is in the same range with what Robards *et al*. (1999) reported. It can be noted by the ranges that average daily consumption of flavonoids is less than 1 g per day, implying that about a handful of ripe black plum fruits, or half litre of its juice or a tea spoon of seed powder (for example in a cup of tea as a vehicle food) can supply this amount.

Antioxidants interact with DPPH in such a way that they either transfer the electrons or hydrogen atoms to DPPH, in return neutralising the free radical species (Naik, 2003). The level to which the free radical (DPPH) is neutralised is reflected by the percentage inhibition. Because of this neutralisation, the colour of the reaction mixture changes from purple to yellow. This results in the decrement of its absorbance at wavelength of 517 nm.

It is shown that the percentage inhibition was increasing in the order; seed powder> pulp >ready-to-drink juice, thus proportional to the concentration of total antioxidants.

Benherlal and Arumughan (2007) reported that some of the various antioxidants in black plum include garlic acid, tannins, anthocyanin, glucoside, malvidin and other components.

Carotenoids

The levels of carotenoids in black plum pulp, seed powder and ready- to drink - juice were not significantly different. According to findings by Swami *et al.* (2012), the content of carotene in the *S. cumini* fruit was 0.004 mg 100 g^{-1} (equivalent to 4.0 µg 100^{-1}) which is closely related to values from this research. These amounts are low and so *S. cumini* is not a good source of carotenoids. It should also be noted that carotenoids can be destroyed by processing, for example drying in light. Like other antioxidants, there are no established dietary allowances for carotenoids (Voutilainen *et al*., 2006). Higdon (2004) also concluded that the existing evidence was insufficient to establish a recommended dietary allowance (RDA).

Vitamin C

Findings from Shahnawaz *et al*. (2009) for vitamin C levels in pulp, dried seed powder and fresh ready-to-drink juice were close to findings from the present study. Both research findings show that the pulp contains the highest level of vitamin C followed by the juice and lastly seed powder.

Sugar composition

The results of this study showed that there are both reducing and non reducing sugars in the fruit pulp and seeds. This is in agreement with what Shahnawaz *et al*., (2009). This gives the fruit the sweet taste. In comparison with values obtained by Shahnawaz *et al*. (2009) for pulp and seed powder in the order 14.31 and 3.64%; total sugars, 5.72 and 1.41 %; reducing sugars; and 8.58 and 2.12%; non- reducing sugars. There is a slight difference which may be attributed to differences in conditions in the location where the fruits belong. However, Sewheg and Das (2015) did not mention the presence of non-reducing sugars but noted that the fruit contains reducing sugars. Swami *et al*. (2012) on the other hand reported that the amount of total sugars was equal to that of reducing sugars.

Studies show that the juice from the black plum is about 10-13 ^{O}Brix (Santhalaksshmy *et al*., 2015) and thus sweet enough. According to the information provided by British Dietetic Association (BDA), it is healthier to take fruits compared to foods high in free sugars and fat. BDA regards foods with more than 22.5 g 100 g^{-1} as a high sugar food. However, the black plum pulp which contains the highest level of sugar as per this study is just half (13.2 g 100 g^{-1}). This means that the black plum fruit is categorised as moderate sugar food.

Sensory evaluation of black plum juice

Considering the overall acceptance scores, sample 425 (control) had the highest scores followed by 834 and then 364. According to most of the panellists, samples 364 and to a lesser extent 834 were much tainted with strong tartness, and astringency thus making them harsh to taste. In their description, some compared them to sour herb taste. In contrast, sample 425 (control) was noted to give a clean taste. Therefore, these unpleasant characteristics stemmed from the addition of the seed powder in the samples 834 and 364. However, in addition to containing higher levels of antioxidants, in attributes like colour (appearance) and thickness (consistence), samples 834 and 364 had relatively higher scores and this was due to the seed powder included in them.

Black plum seed powder is known to contain alkaloids; substances that are known to be bitter. In order to utilise the antioxidants in the seeds, a different vehicle food for example tea can be used. It could also be mixed in flour for making baked products like cookies and biscuits (Kalse *et al*., 2016). When seed powder is added in dough for baked products, on top of obtaining antioxidants, one would obtain fibre if they consumed a *S. Cumini* seed powder enriched baked products (Priyanka *et al.,* 2015). In baked products, the characteristic taste of the seed can be strongly masked. Alternatively the attributes of the juices from sample enriched with the seed powder need to be treated in such a way that can mask the negative unpleasant characteristics.

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