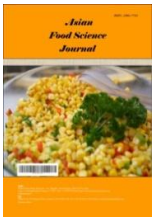

Asian Food Science Journal

18(1): 43-52, 2020; Article no.AFSJ.60169
ISSN: 2581-7752

Phytochemical, Nutritional, Antioxidant Activity and Sensorial Characteristics of Amala (*Phyllanthus emblica* L.) *Chutney*

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Authors' contributions

This work was carried out in collaboration among all authors. Author YKC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SR and AD managed the laboratory analysis of the study. Author SR conducted survey in many localities to collect the information and managed literature searches. Author LDS performed statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/AFSJ/2020/v18i130209
Editor(s):
(1) Dr. Manvesh Kumar Sihag, Mansinhbhai Institute of Dairy and Food Technology, India.
Reviewers:
(1) Sagar Mahendrabhai Bhinde, Gujarat Ayurved University, India.
(2) Prashant Sahni, Punjab Agricultural University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60169

Original Research Article

Received 10 June 2020
Accepted 17 August 2020
Published 17 September 2020

ABSTRACT

This study was aimed to prepare amala (*Phyllanthus emblica* L.) *chutney* and to determine its phytochemicals and nutritional compositions, antioxidant activity and sensorial properties. The amala pulp and sugar were mixed separately at the proportion of 70:30, 60:40, 50:50, 40:60 and 30:70 and labeled as samples A, B, C, D and E respectively. Sample A exhibited highest tannins, total polyphenols, flavonoids content and percent DPPH inhibition (198.9 mg GAE/g, 606 mg GAE/g, 153.47 mg QE/g and 61.67% respectively), and sample B exhibited highest ascorbic acid content (325.4 mg/100g) among the *chutney* samples. The crude proteins, crude fat, crude fiber, total ash and moisture content were higher (2.1%, 0.328%, 5.03%, 1.73% and 51.17% respectively) in sample A. The carbohydrate content and energy value were higher (66.16% and 267.9 Kcal/100 g respectively) in sample E. Total sugar, TSS and pH (75.93%, 60.3 °Bx and 4.56

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respectively) was higher in sample E while acidity (1.21% as citric acid) was high in sample A. Most of the sensory attributes were significantly higher (P<0.05) in sample C, signifying to use the equal proportion of pulp and sugar for the preparation of amala *chutney*.

Keywords: Amala; chutney; phytochemicals and nutritional compositions; antioxidant activity; sensorial properties.

1. INTRODUCTION

Amala (*Phyllanthus emblica* L.) an indigenous fruit in tropic and subtropical areas of Nepal and southeast Asia is a good source of ascorbic acid, polyphenols, flavones, tannins and other bioactive compounds [1]. Amala which is called gooseberry in english provides sufficient nutrition and therapeutic values. It is sour in taste but its acceptability can be increased with various processing techniques. Owing to its excellent nutritional profile and therapeutic properties, it is processed into different types of products. Different products of amala like *murabba*, juice, jam, cheese, candy, powder, beverage, *chutney*, leather etc are preferred by the consumers being the rich source of vitamin C and antioxidants [2]. Amala is one of the main constituents of many ayurvedic preparations like Triphla and Chyawanprash [3-5]. *Chatni* also called as *"chutney"* is a condiment mostly used in Nepal and the Indian subcontinent made of fruits and/or vegetables, sugar, salt, spices and herbs. The product is prepared from peeled, sliced or grated unripe or semi-ripe fruits and /or vegetables by cooking the shredded part with salt over medium heat for 5-10 min (depending upon the volume, vessel size and nature of raw materials), mixing and then adding sugar, spices and vinegar then filled hot in sterilized jars. The preparation is much more similar to jam except spices and vinegar is added. In some localities of Nepal such preparations are called "*Fatke Chatni"* which means cooking until it gives vigorous cooking sound. In indigenous localities like *Tharu, Rajbanshi, Satar* in Terai regions and even in *Brahmin* and *Chhetri* community in hilly regions of Nepal "*Chatni*" is famous condiment [6-8]. "*Chatni*" comes from Nepali word-"*Chatne"* which means licking. As per *Tharu* and *Rajbanshi* people, *chatni* is a condiment which is prepared by grinding (especially in "*loro"*- stone tool to grind and "*silauto"*-flat stone base where raw materials are kept) spices, salt, vinegar and any unripe fruits. When *Maithili* and *Bhojpuri* people of Nepal were asked about *chutney* they called it "*Khatta-Mitthi*" (i.e, sweet-sour). So the *chutney* has different names and way of preparation varying with community, culture and geography. A lot of researches have been conducted depending upon the ethno-cultural variations. Curry leaf *chutney* powder [9], mango *chutney* [10], guava *chutney* [11], amala *chutney* [12] and guava- papaya *chutney* [13] were studied earlier. Excess amount of harvested amalas can be turned into higher value products with increased self-life. Similarly, amala has astringent and acidic taste and flavor naturally. Preparation of amala *chutney* increases the sensorial acceptability of amala and its market value. There are limited studies regarding the development of ready to eat Amala *chutney* in Nepal. So, present study is concerned with the effort to evaluate the phytochemicals and nutritional compositions, antioxidant activity and sensorial properties of the amala *chutney*.

2. MATERIALS AND METHODS

2.1 Collection of Raw Materials

Amala (*Phyllanthus emblica* L.) was obtained from, Pakhribas (27°03'12.3"N,87°16'37.6"E, elevation 1667 m), Dhankuta, hilly region of Nepal. Sugar, salt and spices were obtained from the market of Itahari, Nepal.

2.2 Preparation of *Chutney*

Chutney was prepared as described by many researchers [10-13] with some modifications as per the taste and practices of local people. Damaged and bruised fruits were sorted out. Large and medium amalas were selected. The selected fruits were washed with tap water to remove dusts, and adhered impurities. Sound washed amalas were boiled in water for 5 minutes. Water was immediately drained off and cooled with tap water. The fruits were then cut through ridges and seeds were removed. The cut fruit pieces were grinded to prepare pulp with addition of water (10 mL per 100 g). Dried spices were brought and grinded to prepare spice mix. Cinnamon 8 g, small cardamom 8 g, cloves 4 g, black pepper 5 g, chilly 3 g, ginger powder 2 g, bay leaves, 5 g, cumin 2.5 g and coriander 2.5 g were mixed and grinded. Pilot scale study was carried out and only 2% of the spice mix was

added in all preparations. In a low flame (~ 70-75°C), the pulp was cooked in a stainless steel with addition of sugar, spices mix and salt. Continuous stirring was done while cooking until the desired uniform consistency was obtained. The products were poured hot into clean, dry and sterilized glass jars. Jars were corked after cooling and stored airtight. Proportion of amala pulp and sugar was varied for different preparations (Table 1).

2.3 Proximate and Chemical Analysis

Proximate analysis of raw materials was carried out in triplicate. Standard AOAC methods: (AOAC 935.29) for moisture content, (AOAC 922.06) for crude fat , (AOAC 992.23) for crude protein, (AOAC 923.03) for total ash and (AOAC 962.09) for crude fiber were used [14].Total carbohydrate in all raw materials was calculated by deducting predetermined values of moisture, crude fat, crude protein and total ash from 100. The energy values were calculated by multiplying the values of crude proteins, lipids, and carbohydrates by recommended factors (4, 9, and 4, respectively). The energy values were expressed as Kcal/100 g [15]. Ascorbic acid (vitamin C) was determined by 2, 6 - dichlorophenol indophenol visual dye method [16]. The TSS of the amla pulp and prepared fruit *chutney* was determined by using hand refractometer (AviChem Industries, India). For the determination of pH, digital pH meter (± 0.1 units, Hanna, Mauritius) was used. Acidity was determined by titrimetric method and total sugar was determined by using Fehling's solutions [16].

2.4 Extract Preparation

The pulp and *chutney* were subjected for phytochemicals extraction using methanol [17]. Briefly, 10 g of powdered samples were steeped in 100 mL of 80% methanol for 12 h at room temperature. Then, these samples were filtered using whatman filter paper (No. 41). After filtration, all the extracts were stored in screw capped bottles at 2–4°C until further analysis. The concentration of the extract was determined by evaporating 10 mL of extract to dryness (at 80°C) and measuring the weight of the residue.

2.5 Determination of Total Phenol Content

Total phenolic content (TPC) was determined using spectrophotometric method [18] with some modifications. The reaction mixture was prepared by mixing 0.5 mL of plant extract solution, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% of Na_2CO_3 aqueous solution. The samples were thereafter incubated in a thermostat at 45ºC for 45 min. The absorbance was determined using spectrophotometer at wave length of 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GAE/g of extract).

2.6 Determination of Total Flavonoid Content

Total flavonoid content was determined using a modified aluminium chloride assay method [19]. Two mL of solution was pipette out in a test tube in which 0.2 mL of 5% sodium nitrate ($NaNO_3$) was mixed and rested for 5 min. Then, 0.2 mL aluminium chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 min. Add 2 mL of 1N sodium hydroxide (NaOH) in the tube and finally volume was made up to 5 mL. The absorbance was measured after 15 min at 510 nm against a reagent blank. The test result was correlated with standard curve of quercetin (20, 40, 60, 80, 100 µg/mL) and the total flavonoid content was expressed as mg quercetin equivalents (mg QE/g of extract).

Table 1. Coding of the samples with different proportions of amala pulp and sugar

Samples	Amala Pulp (parts)	Sugar (parts)	Spices mix (%)	Salt (%)
A	70	30	2	2
B	60	40	2	2
C	50	50	2	2
D	40	60	2	2
E	30	70	2	2

2.7 Determination of Tannins

Tannins were determined by Folin-Ciocalteu method [20]. In brief, 0.1 mL of the sample extracts added to volumetric flask (10 mL) containing 7.5 mL distilled water and 0.5 mL Folin-Ciocalteu reagent, 1 mL 35% Na_2CO_3 solution and dilute to 10 mL distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solution of gallic acid (20, 40, 60, 80, 100 µg/mL) was prepared in same manner as described earlier. Absorbance for test and standard solution was measured against blank at 725 nm with an UV/visible spectrophotometer. The tannin content was expressed in terms of gallic acid equivalent (mg GAE/g of extract).

2.8 Determination of DPPH Radical Scavenging Activity

DPPH free radical scavenging activity of extracts was determined by spectrophotometric methods [21]. Different dilutions of the extracts were made using 80% methanol (4 mg of DPPH in 100 mL methanol to give a solution of 100 µM). Then, 1 mL of the extract was mixed with 2 mL of 0.1 mMDPPH solution. The absorbance was read at 517 nm after 30 min incubation in the dark. Finally, percentage scavenging activity was determined using following equation:

$$\% \text{ scavenging activity} = (Ac\text{-}As) \times 100 / Ac$$

Where, Ac refers to absorbance of control and As refers to absorbance of test sample.

2.9 Sensory Evaluation

Ten panelists of 20-35 years with sound health were trained before evaluating the samples of amala *chutney* by using 9-point hedonic rating test [16]. A 2 h training session was conducted for 4 days to familiarize panel members with sensory attributes and sensory evaluation was conducted between 1:00 PM to 2:00 PM. The panelists were provided with the uniform quantity of *chutney* in stainless steel plate to analyze color, flavor, taste, mouth-feel and overall acceptability.

2.10 Statistical Analysis

The data of each experimental analysis that were performed in triplicate was analyzed by one- way analysis of variance (ANOVA) by using software GenStat Release 12.1 (Copyright 2009, VSN International Ltd.). Means were compared using Tukey's HSD post hoc test ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Nutritional and Chemical Composition of the Fresh Amala Fruit

Proximate composition and chemical composition of the amala fruit is presented in Table 2 and Table 3. Moisture content of the amala was found higher than in the study of Iwansyah et al. [22] but it was similar to the findings of Parveen and Khatkar [23]. Total ash, crude protein, crude fiber and carbohydrate of the amala were similar to the findings of Iwansyah et al. [22] but in the study of Parveen and Khatkar [23], crude fiber was found higher. Some of the parameters were similar and some were different when compared to the study of Khattak [24]. The pH and TSS was similar to the findings of some researchers [25,26]. Ascorbic acid content of 573 mg/ 100 g was reported. Fruits and vegetables are rich in vitamins and polyphenols. Although amla fruit is one of the richest sources of ascorbic acid (445–468 mg of vitamin C/100 g amala fruit) and has many phenolic compounds, but the sour and astringent taste makes the fresh amala fruit less acceptable to consumers [27]. Total sugar reducing and non-reducing sugars were less than that of findings of Parveen and Khatkar [23] but higher than the findings of Ranote et al. [26].Total polyphenol content (TPC) and total flavonoid content (TFC) was found less than the findings of Alkandari et al. [28] but higher than the findings of Ruangchakpet and Sajjaanantakul [29]. Tannin content in the amala fruit was found to be 245.49 mg GAE/g. which was higher than reported by Khattak [24]. The DPPH radical scavenging activity was similar to findings of some researchers [30,31]. The variation in proximate and chemical composition is contributed by variation of variety, soil profile, climate, geography, cultivation methods and climate. Variation of phytochemicals might be due to the variation of the extraction solvents. Spice mix is prepared from nine varieties of spices which have different phytochemical characteristics. The content of tannin, flavonoids, total polyphenols and percent DPPH inhibition of the spice mix in presented in Table 3.

Table 2. Nutritional analysis of amala fruit

Chemical composition	Values
Moisture (%)	82.76 ± 1
Total Ash (%)	2.3 ± 0.2
Crude Fat (%)	0.28 ± 0.13
Crude protein (%)	2.03 ± 0.2
Crude fiber (%)	5.1 ± 0.45
Total Carbohydrate (%)	7.58 ± 1.27
Total Energy (Kcal/ 100 g)	41 ± 6.5

Values are means of triplicate ± standard deviations

Table 3. Chemical, phytochemical analysis and anti-oxidant activity of amala fruit and spice mix

Chemical and phytochemical content	Amala fruit	Spice mix
Acidity (% citic acid)	1.8 ± 0.1	------
pH	2.96 ± 0.2	------
TSS (°BX)	11.7 ± 0.5	------
Ascorbic acid (mg/100 g)	573 ± 19	------
Reducing sugar (%)	3.94 ± 0.9	-----
Non reducing sugar (%)	9.08 ± 0.77	-----
Total sugar (%)	13.02 ± 1.68	-----
Total Polyphenol content (mg GAE/g)	730.15 ± 10.49	650.75 ± 6.4
Tannin content (mg GAE/g)	245.49 ± 6.69	210.49 ± 4.61
Flavonoid content (mg QE/g)	161.83 ± 11.21	135.3 ± 7.35
Percent DPPH inhibition (%)	67.02 ± 1.06	59.13 ± 2.11

Values are means of triplicate ± standard deviations

3.2 Chemical, Phytochemical Analysis and Antioxidant Activity of *Chutney*

The ascorbic acid contents were highest (325.2 mg/100 g) in sample B (containing 60 parts amala pulp) and the lowest (59.3 mg/100 g) in sample E (containing 30 parts amala pulp). Similarly TPC, TFC, percent DPPH inhibition and tannin content in the *chutney* was found to be in the range of 606-136.4 mg GAE/g, 153.47-39.67 mg QE/g, 61.67-26.37% and 198.9-65.6 mg GAE/g respectively. TPC, TFC, percent DPPH inhibition and tannin content of sample A was higher than other samples. The antioxidant activity of the extracts can be assessed by employing DPPH free radical scavenging activity [15]. The TPC, TFC, percent DPPH inhibition and tannin content in the *chutney* gradually increased with the increase of the amala pulp in the *chutney* (Table 5). With respect to phytochemical content of fresh amala (Table 3) the *chutney* prepared from the different proportion of amala pulp was found higher. This might be due to the addition of the spices which are also rich in phytochemicals (Table 3). The added spices: cinnamon [32] small cardamom [33], cloves [34], black pepper [35], cumin [34,36], coriander [37], bay leaves [38], dried ginger [39] and chilly powder [40] were found to be rich in phytochemicals and antioxidant activity. These spices might have caused increased in the phytochemicals and antioxidant activity of amala *chutney* samples. *Chutney* samples with higher proportion of amala had higher content of vit. C. The vit C was varied in the amala *chutney* samples as per the proportion of amala pulp addition. Decrease in ascorbic acid was observed which might be due to exposure in temperature and open environment for longer time during *chutney* preparation [41,42]. Total phenol content in the *chutney* samples were found to be related significantly ($P<0.05$) with antioxidant activity ($y = 0.0701x + 19.485$ $R^2 = 0.9396$). Thus, higher antioxidant activity of the samples was contributed by the *chutney* samples with higher TPC. Higher amount of polyphenols, antioxidant and ascorbic acid was reported in amala products [12,28]. Lower acidity and higher pH was reported with the decrease in the amala pulp proportion in the *chutney* samples (Table 6). Total sugar and TSS were found to increase with increase in sugar proportion (Table 6). The acidity, pH sugar and TSS in the fresh amala (Table 3) contributed to the respective parameters in *chutney* with different proportions of amala pulp and sugar.

Table 4. Nutritional analysis of different samples of amala *chutney*

Samples	Moisture content (%)	Crude fat (%)	Crude protein (%)	Crude fiber (%)	Carbohydrate (%)	Total ash (%)	Energy (Kcal/100 g)
A	51.17 ± 0.97[a]	0.328 ± 0.01[c]	2.1 ± 0.1[d]	5.03 ± 0.15[d]	39.63 ± 0.81[a]	1.738 ± 0.04[a]	169.9 ± 3.38[a]
B	48.3 ± 0.7[b]	0.288 ± 0.02[bc]	1.42 ± 0.02[c]	3.1 ± 0.06[c]	47.41 ± 1.27[b]	1.51 ± 0.04[b]	197.9 ± 5.39[b]
C	46.2 ± 1.7[c]	0.26 ± 0.02[b]	0.69 ± 0.1[b]	1.8 ± 0.02[b]	55.93 ± 1.7[c]	1.193 ± 0.07[c]	228.8 ± 7.21[c]
D	45.53 ± 0.7[d]	0.16 ± 0.01[a]	0.61 ± 0.02[ab]	1.35 ± 0.05[a]	61.93 ± 1.1[d]	0.813 ± 0.01[d]	251.7 ± 4.22[d]
E	43.4 ± 1.41[e]	0.12 ± 0.01[a]	0.55 ± 0.02[a]	1.29 ± 0.01[a]	66.16 ± 0.39[e]	0.24 ± 0.02[e]	267.9 ± 1.72[e]

Values are means of triplicate ± standard deviations. Values in the columns bearing the different superscripts are significantly different (P<0.05)

Table 5. Phytochemical analysis and antioxidant activity of different samples of amala *chutney*

Samples	Ascorbic acid (mg/100 g)	Total Polyphenol content (mg GAE/g)	Tannin content (mg GAE/g)	Flavonoid content (mg QE/g)	Percent DPPH inhibition (%)
A	297.9 ± 2.1[a]	606 ± 5.95[a]	198.9 ± 1.1[a]	153.47 ± 1.36[a]	61.67 ± 0.67[a]
B	325.2 ± 4.7[b]	515.7 ± 5.46[b]	179.4 ± 3.1[b]	111.37 ± 1.23[b]	54.63 ± 1.25[a]
C	197.6 ± 2.55[c]	351.4 ± 5.6[c]	113.1 ± 3.5[c]	66.57 ± 2.13[c]	45.9 ± 1.15[b]
D	121.3 ± 0.6[d]	214.8 ± 5.29[d]	86.4 ± 2.16[d]	54.2 ± 1.05[d]	36.83 ± 1.6[c]
E	59.3 ± 3.11[e]	136.4 ± 3.1[e]	65.6 ± 2.5[e]	39.67 ± 2.51[e]	26.37 ± 6.38[d]

Values are means of triplicate ± standard deviations. Values in the columns bearing the different superscripts are significantly different (P<0.05)

Table 6. Chemical analysis of different samples of amala *chutney*

Samples	TSS (°Bx)	Total sugar (%)	Acidity (%)	pH
A	25.67 ± 1.52[a]	33.88 ± 1.12[a]	1.21 ± 0.01[a]	3.06 ± 0.11[a]
B	35.33 ± 0.57[b]	46.23 ± 1.07[b]	0.9267 ± 0.02[b]	3.53 ± 0.15[b]
C	44 ± 1[c]	55.3 ± 1.47[c]	0.79 ± 0.02[c]	3.8 ± 0.1[b]
D	54 ± 1[d]	66.33 ± 2.08[d]	0.7067 ± 0.01[d]	4.16 ± 0.12[c]
E	60.33 ± 0.57[e]	75.93 ± 0.92[e]	0.6533 ± 0.01[d]	4.56 ± 0.16[d]

Values are means of triplicate ± standard deviations. Values in the columns bearing the different superscripts are significantly different (P<0.05)

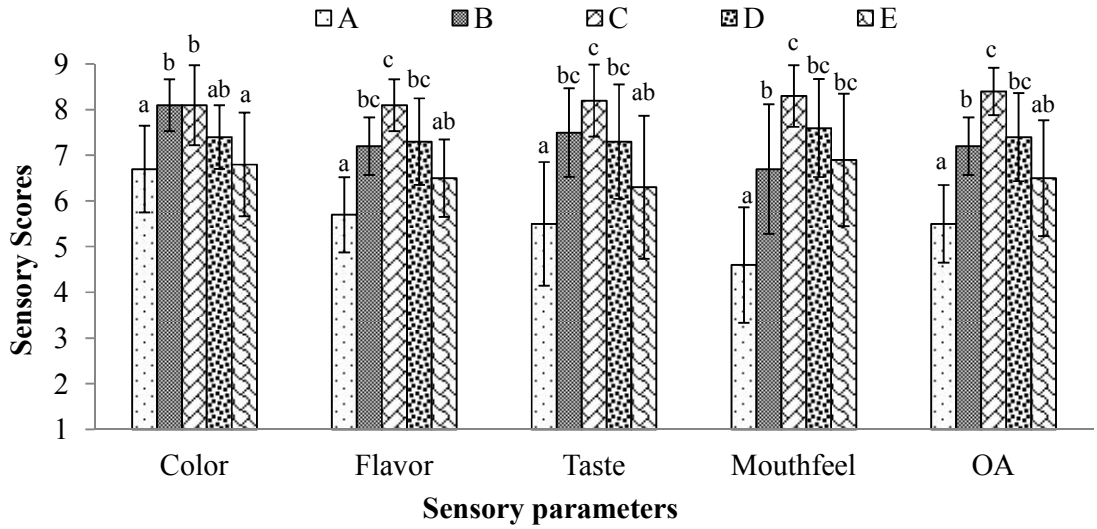

Fig. 1. Sensory analysis of the different samples of *chutney*. Error bars show standard deviation and error bars bearing different superscript differs significantly (P<0.05)

3.3 Nutritional Analysis of *Chutney*

Crude fat, crude protein crude fiber, total ash, moisture and energy content of the different *chutney* samples varied significantly (p<0.05) and found to be in the range of 0.12-0.32%, 0.55-2.1%, 1.29-5.03%, 0.24-1.73%, 43.4-51.17% and 169.9-267.9 Kcal/100 g respectively (Table 4). The content of proximate components was reported according to the proportion of amala pulp addition. The slight changes were observed which might be due to addition of spice mix and effect of processing conditions. The moisture content was found to be increased with increase in amala pulp proportion. Higher amount of sugar might have caused dehydration effecting resulting in the moisture reduction [43]. Energy value of sample E (*chutney* with 30 part amala pulp) was found higher than others which might be due to contribution of higher amount of sugar [44].

3.4 Sensory Analysis of *Chutney*

Sensory parameters color, flavor, taste, mouthfeel and overall acceptability of the samples of *chutney* varied significantly (Fig. 1). *Chutney* with higher part of amala (sample A) was not liked by many panelists. Astringent and acidic taste and flavor in sample A might be due to more amala pulp. Amala has astringent and acidic taste and flavor naturally [45]. Astringency and sourness due to more pulp might be the reason of low mouthfeel in sample A. Overall acceptability (OA) of sample C was significantly higher than other samples (Fig. 1). Sample C (*chutney* with 50 parts amala and 50 parts pulp) was found superior in sensory characteristics. This might be due to the balance of sugar and pulp where extreme astringency and sourness were masked. The sensory evaluation, here found that the desirable characteristics, greenish red color, pleasant flavor, taste, aftertaste and overall acceptability were higher in sample C, suggesting that equal proportion of pulp and sugar was more desirable in *chutney* preparation.

4. CONCLUSION

Chutney is a condiment mostly used in Nepal and Indian subcontinent that is made of fruits and vegetables, sugar, salt, spices and herbs. Amala is astringent and sour, making it less acceptable to consume it as raw fruit. Preparing *chutney* from amala increases the sensorial acceptability of amala and its market value. Phytochemicals and nutritional compositions, antioxidant activity and sensorial properties of amala *chutneys* prepared at different ratio of pulp and sugar were studied. Amala *chutney* with higher amount of pulp (70 parts) was found to have significantly higher (P<0.05) content of phytochemicals and nutritional attributes. However, most of the sensory characteristics were significantly high (P<0.05) in sample C (*chutney* prepared from equal proportion of pulp and sugar). Though the *chutney* with more pulp was higher in antioxidant and phytochemicals content, it is suggested to use equal proportion of sugar and pulp in

chutney preparation for higher consumer acceptance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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