

## Original Research Article

### **Determination of functional properties of Sri Lankan Ambarella (*Spondias dulcis* Forst. syn. *Spondiascytherea* Sonn.) fruit and development of value-added products**

#### **Abstract**

**Objective:** To develop vacuum dried (VD) Ambarella fruit powder and Ambarella fruit incorporated soup mix from large and miniature **Amberrella** and investigation of antioxidants and anti-diabetic properties.

**Methods:** Ethanolic extracts of two Ambarella varieties were analyzed for antioxidant potential in terms of total phenolic content (TPC), total flavonoid content (TFC), ferrous reducing antioxidant power (FRAP), radical scavenging activities of DPPH and ABTS and  $\alpha$ -amylase inhibitory activity.

**Results:** Fresh dwarf Ambarella showed a significantly ( $p < 0.05$ ) high TPC ( $3.35 \pm 0.10$  mgGAE/g) while fresh large Ambarella showed a significantly ( $p < 0.05$ ) high FRAP ( $0.71 \pm 0.13$  mgTE/g) and DPPH ( $3.57 \pm 0.31$  mg TE/g). In comparison of ethanolic extracts of VD Ambarella powders, a significantly ( $p < 0.05$ ) higher antioxidant potential in terms of FRAP ( $4.19 \pm 0.06$  mgTE/g) exhibited in dwarf variety while significantly ( $p < 0.05$ ) higher ABTS ( $4.03 \pm 0.27$  mgTE/g) and DPPH ( $3.00 \pm 0.49$  mgTE/g) exhibited in large Ambarella. Alpha amylase inhibition activities of fresh fruits of large and dwarf were  $46.30 \pm 4.07\%$  and  $49.55 \pm 3.18\%$  where as in VD powders were  $27.59 \pm 5.03\%$  and  $15.58 \pm 5.86\%$  respectively. An instant soup mixture was developed incorporating 20% of VD powder from large variety due its abundance. The antioxidant potential of ethanolic extract of the soup mixture in terms of TPC, TFC, ABTS, and FRAP were  $0.55 \pm 0.00$  mgGAE/g,  $0.04 \pm 0.00$  mgQE/g,  $1.65 \pm 0.06$

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mgTE/g, and  $0.04 \pm 0.15$  mgTE/g. Alpha amylase inhibition activity of soup mixture was  $39.49 \pm 0.29\%$ .

**Conclusion:** The both types of fresh fruits exhibited higher antioxidant potential except FRAP and higher anti-amylase inhibition than VD Ambarella powder.

**KEY WORDS:** *Spondiasdulcis*, functional properties, instant soup mixture, Antioxidant potential

**Comment [A2]:** I suggest deleting this word "functional properties" because it is present in the title and insert "  $\alpha$ -amylase enzyme inhibition properties"

## 1. INTRODUCTION

There are two distinct types of Ambarella (*Spondiascytherea*) fruits; the large type, which is more popular, and the dwarf type or the miniature. However, dwarf type Ambarella fruit remains unexploited in Sri Lanka due to the several advantages over the large fruit type [1].

Ambarella fruit exhibits high pH value 3.87-4.05 and total titratable acidity 0.47% [2]. Fruit contains moisture 59.65% to 85.47%, protein 0.50% to 0.80%, fat 0.28% to 1.79%, sugar 8.05% to 10.54% and crude fiber 0.85% to 3.60%. Potassium, phosphorus and calcium are the main minerals found in the fruit while Vitamins C (42 mg/100 g) and Vitamin A are the main vitamins found [2]. The versatility usage of both types of Ambarella fruits is reflected in the broad range of value-added products including amchar, sweet and sour pickles, chutney, jams, jellies, alcoholic and non-alcoholic beverages, syrups, nectars and sauces [1-3].

Recently, the consumption of Ambarella is increasing based on its nutraceutical properties against diabetes mellitus, indigestion, urinary tract infections, hypertension and hemorrhoids [1,2,4].

At present, the research approaches on plant antioxidants have received more attention to combat chronic diseases caused by oxidative stress such as cancer, alzheimer's and diabetes due to the possible toxicity of synthetic antioxidant [5,6]. The present study was aimed to analyze the antioxidant potential and type II diabetes related  $\alpha$ -amylase enzyme inhibition properties in two types of Ambarella grown in Sri Lanka.

## **2. MATERIALS AND METHODS**

### **2.1 Materials**

#### **Fruits**

Mature unripe Ambarella (*Spondias dulcis* Forst. syn. *Spondiascytherea* Sonn) fruits obtained from local Supermarket at Malabe, Sri Lanka.

#### **Chemicals and reagents**

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazine (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), potassium persulfate, ferric chloride, quercetin, soluble starch, Folin-Ciocalteu phenol reagent and 3,5-dinitrosalicylic acid (DNS) were purchased from Sigma-Aldrich, USA. All the other chemicals used were of analytical grade.

#### **Enzymes**

Alpha-Amylase from *Bacillus* sp. (*Bacillus licheniformis*), Novozymes A/S, was purchased from Denmark, Sigma Aldrich.

### **2.2 Methods**

#### **2.2.1 Preparation of VD powder**

Fresh fruits were washed properly and then soaked in 100 ppm Clorox solution for 4-5 min. Edible part was cut into thin pieces and dipped in 0.2% citric solution. The cut pieces were washed and subjected to steam blanch for 1 min. They were dried in vacuum dryer for 6 hours at 60 °C and ground to pass through 0.5 mm sieve.

#### **2.2.2 Preparation of fresh fruit pulp**

The edible portion (i.e. without peel and seeds) of the fruit was thoroughly crushed using dry grinder (Prestige Deluxe LS 304).

#### **2.2.3 Extraction for determining functional properties**

25.00 g of each fresh fruit pulp from two varieties and 2.00 g of each VD powders from two varieties were separately shaken over night using orbital shaker (110 rpm) at room temperature ( $28 \pm 2^\circ\text{C}$ ) with 4 times of the sample weight of 80% ethanol. Extracts were then filtered and ethanol was evaporated in a rotary evaporator. Freeze-dried extracts were collected into the eppendorf tubes separately and labeled. They were stored at  $< 0^\circ\text{C}$ .

## **2.2.4 Determination of Antioxidants**

### **2.2.4.1 Total Polyphenolic Content (TPC)**

The TPC of fresh pulp and the VD powder were carried out as method described by Singleton et al., 1999 [7].

Method in briefly, each extract was diluted in distilled water (2 mg/ml). 20  $\mu\text{L}$  of sample, 110  $\mu\text{L}$  of 10 times diluted Folin-Ciocalteu reagent and 70  $\mu\text{L}$  of 10% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution were mixed in a well of 96-well micro plate. After incubating 30 min at  $25 \pm 2^\circ\text{C}$ , absorbance was measured at 765 nm using a micro plate reader (SpectraMax Plus 384, Molecular Devices, USA) using gallic acid as the standard.

TPC was expressed as mg gallic acid equivalents (GAE) / g of the sample on dry weight basis.

### **2.2.4.2 Total Flavonoids of Content (TFC)**

The TFC of fresh pulp and the VD powder were carried out as method described by Pourmorad, 2006 [8].

Method in briefly, each extract was diluted in methanol (2 mg/ml). 100  $\mu\text{L}$  diluted sample and 100  $\mu\text{L}$  of 2% aluminium chloride were added into a well of 96-well micro plate. After incubating 10 min at  $25 \pm 20^\circ\text{C}$ , the absorbance was measured at 415 nm using quercetin as the standard using micro plate reader (SpectraMax Plus 384, Molecular Devices, USA).

The results were expressed as mg quercetin equivalents (QE) /g of the sample on dry weight basis.

## **2.2.5 Determination of Antioxidant Potential**

### 2.2.5.1 DPPH Assay

The DPPH radical scavenging activity of fresh pulp and the VD powder were carried out as method described by Blois, 1958 [9].

Method in briefly, 125  $\mu\text{L}$  of DPPH radical (20 mg/100 ml) and 50  $\mu\text{L}$ (2 mg/ml) of sample were mixed in a well and incubated  $25 \pm 2^\circ \text{C}$  for 10 min. Absorbance was measured 517 nm using micro plate reader (SpectraMax Plus 384, Molecular Devices, USA).

Activity in term of Trolox equivalents (TE) / g for each sample was calculated using Trolox standard curve.

Selected high activity extracts were run for the dose response studies at concentrations of 9.375, 18.75, 37.5, 75, 150, 300  $\mu\text{g}/\text{mL}$  and  $\text{IC}_{50}$  values for extracts were calculated using a graph (activity vs. extract concentration).

DPPH radical scavenging activity (%) =  $[(\text{Ac} - \text{As}) / \text{Ac}] * 100$  where, Ac is the absorbance of the control and As is the absorbance of the sample.

### 2.2.5.2 ABTS Assay

The ABTS radical scavenging activity of fresh pulp and the VD powder were carried out as method described by Re et al., 1999 [10].

40  $\mu\text{L}$  of seven times diluted ABTS stock solution (10 mg of ABTS in 2.5 ml of 2.5 mM potassium persulphate solution incubating at  $37^\circ \text{C}$  for 16 h in dark), 110  $\mu\text{L}$  phosphate buffer and 50  $\mu\text{L}$  (2 mg/ml) of sample was incubated at  $25 \pm 2^\circ \text{C}$  for 10 min. Absorbance was recorded at 734 nm using micro plate reader (SpectraMax Plus384, Molecular Devices, USA).

Activity in term of Trolox equivalents (TE) / g for sample was calculated using Trolox standard curve.

Selected high activity extracts were run for the dose response studies at concentrations of 7.81, 15.62, 31.25, 62.5, 125, 250  $\mu\text{g}/\text{mL}$  and  $\text{IC}_{50}$  values for extracts were calculated using a graph (% activity Vs. extract concentration).

ABTS radical scavenging activity (%) = [(Ac - As) / Ac]\*100 where, Ac is the absorbance of the control and As is the absorbance of the sample.

### 2.2.5.3 Ferric Reducing Antioxidant Power (FRAP) assay

Ferric reducing antioxidant power (FRAP) of fresh pulp and the VD powder were carried out as method described by Benzie & Szeto, 1999 [11].

Method in briefly, 150 µL of FRAP reagent (mix containing 300 mM of acetate buffer at pH 3.6, 10mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40mM HCl solution and 20mM FeCl<sub>3</sub>.6H<sub>2</sub>O in a ratio of 10:1:1 followed by incubation at 37°C for 10min), 30 µL acetate buffer and 10 µL (2 mg/ml) were transferred to a micro well. After incubating at 25 ± 2°C for 10 min, absorbance was measured at 600 nm via micro plate reader (SpectraMax Plus 384, Molecular Devices, USA).

Results were expressed as mg Trolox equivalents (TE)/ g of the each sample on dry weight basis.

### 2.2.6 Anti-amylase Assay

The anti-amylase assay of fresh pulp and the VD powder were performed according to the method described by Premakumara *et al.* 2013 [12].

50 µl of sample (2 mg/ml), 40 µl of starch (1% w/v) and 860 µl of 100 mM Sodium Acetate buffer were incubated at 40 °C in shaking water bath for 10 min. For control, 40 µl of starch and 910 µl of buffer and for blank 950 µl of buffer were added and incubated at 40°C in shaking water bath for 10 min. 50 µl of working enzyme solution (2 mg/ml of Alpha-Amylase) was added to sample and control except for blank which 50 µl of buffer solution was added instead of working enzyme solution. Content was incubated at 40 °C in shaking water bath for 15 min. Then 500 µl of 3,5-dinitrosalicylic acid (DNS) was added to sample, blank and control and boiled for 5 min. They were cooled in ice bath. 200µl of content was transferred to a well in a 96- well plate and the absorbance was recorded at 540 nm using

**Comment [A3]:** I suggest standardize: Benzie and Szeto, 1999

micro plate reader (SpectraMax Plus 384, Molecular Devices, USA). Anti-amylase activity (% inhibition) of each sample was calculated using the following equation.

$$\text{Inhibition \%} = (A_c - (A_s - A_b) / A_c) * 100$$

Where,  $A_c$  is the absorbance of the control,  $A_b$  is the absorbance produced by sample blank and  $A_s$  is the absorbance the of sample.

### 2.2.7 Development of Ambarella incorporated powdered soup mix

Red rice and green gram were soaked for 2 hours and corn seeds were soaked for more than 5 hours. They were drained and cooked in excess boiled water for 20 min and dried in a dehydrator at 60°C (< moisture content 10%). Samples were ground to particle size less than 0.5 mm and stored in airtight container separately before used. Slices of pumpkin and carrot were steam blanched for 1 min and dried in dehydrator at 60 °C until the material completely dried (moisture content 10%). The dried slices were ground to particle size 0.5 mm and stored in airtight containers separately. Soup mix was prepared by dry mixing of VD powder of large Ambarella, green gram powder, rice flour, corn flour, powdered carrot, powdered pumpkin, garlic powder and salt as shown Table 1. Serving size per person was prepared by adding 10 g of powdered soup mix with 125 ml of boiling water.

**Table 1: Proportion of ingredients used in different formulations of powdered soup mix**

<b>Ingredient</b>	<b>Formula 1 (g / 100g)</b>	<b>Formula 2 (g / 100g)</b>	<b>Formula 3 (g / 100g)</b>
Ambarella powder	15	20	25
Green gram powder	30	25	20
Rice flour	20	20	20
Corn flour	15	15	15
Carrot powder	5	5	5
Pumpkin powder	5	5	5
Garlic powder	5	5	5

Salt	5	5	5
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The three prepared soup samples according to the formula given in Table 1 were subjected to screened sensory panel for sensory evaluation with regard to given sensory characteristics of color, taste, thickness, mouth feel or palatability and overall acceptability. The responses of respondents were recorded using seven-point hedonic scale.

### 2.2.8 Chemical analysis of soup mix

Proximate composition of Ambarella incorporated powdered soup mix was determined according to the methods described in AOAC, 2012 [13].

Analysis was performed moisture content by oven drying at 105 °C for 5 h, fat content by Soxhlet extraction method at 150°C for 2.5 h using petroleum ether (40-60 °C), crude fiber by acid/alkali digestion, ash contents by dry ashing method and crude protein contents by wet digestion followed by steam distillation (kjeldhal method).

Carbohydrate content and energy value of Ambarella incorporated powdered soup mix were determined using a mathematical equations as described by Sompong *et al.*, 2011 [14].

CHO % = 100 - (ash + protein + fat + crude fiber + moisture) %

Energy value (Kcal/100 g) = (% Protein × 4) + (% Fat × 9) + (% Carbohydrate × 4)

Antioxidants, antioxidant potential and anti-amylase activity of soup mix were carried out as methods described in sections 2.2.4, 2.2.5 and 2.2.6.

### 2.2.9 Data analysis

The data of the antioxidant potential of fresh Ambarella and VD Ambarella powder were statistically evaluated by one-way analysis of variance (ANOVA) using Minitab 17 software and significant differences between means were determined by Tukey 's multiple comparison at 5% significant level. The data obtained from the sensory evaluation was statistically

analyzed by using SPSS software with Kruskal-Wallis test method under nonparametric analysis.

### 3. RESULTS AND DISCUSSION

#### 3.1 Determination of functional properties of fresh fruit and VD powders

Vacuum drying is an ideal method of thermal drying for oxygen sensitive materials (e.g. fruits and vegetables) due to the advantage of removing moisture at low temperatures and minimizing the possibility of oxidation reactions [15].

##### 3.1.1 TPC of ethanolic extracts of fresh Ambarella fruit and VD powders

TPC of ethanolic extracts of fresh Ambarella fruit and VD powders are given in Table 2.

**Table 2: Functional properties of fresh fruits of Ambarella varieties and their VD powders**

Functional property	Large Ambarella	Miniature Ambarella	VD large Ambarella	VD miniature Ambarella
TPC (mg GAE/g)	3.05±0.00 <sup>b</sup>	3.36±0.11 <sup>a</sup>	1.49±0.110 <sup>c</sup>	1.25±0.04 <sup>d</sup>
TFC (mg QE/g)	0.12±0.05 <sup>a</sup>	0.16±0.03 <sup>a</sup>	0.07±0.00 <sup>b</sup>	0.08±0.00 <sup>b</sup>
FRAP (mg TE/g)	0.71±0.13 <sup>c</sup>	0.32±0.01 <sup>d</sup>	1.64±0.195 <sup>b</sup>	4.19±0.06 <sup>a</sup>
ABTS (mg TE/g)	3.83±0.16 <sup>a</sup>	3.87±0.16 <sup>a</sup>	4.03±0.274 <sup>a</sup>	3.06±0.09 <sup>b</sup>
(IC <sub>50</sub> µg/ml)	599.9±24.8 <sup>b</sup>	664.7±27.3 <sup>a</sup>	287.4±19.1 <sup>d</sup>	346.47±10.9 <sup>c</sup>
DPPH (mg TE/g)	3.57±0.31 <sup>a</sup>	2.13±0.23 <sup>b</sup>	3.00±0.49 <sup>a</sup>	1.09±0.04 <sup>c</sup>
(IC <sub>50</sub> µg/ml)	734.6±64.4 <sup>b</sup>	1384.9±158.2 <sup>a</sup>	376.7±66.2 <sup>c</sup>	927.5±35.7 <sup>b</sup>
Alpha -AIA				
(% inhibition 100µg/ml of extract)	46.30±4.07 <sup>a</sup>	49.55±3.18 <sup>a</sup>	27.59±5.03 <sup>b</sup>	15.58±5.86 <sup>b</sup>

Data presented as mean ± SD (n=3). Mean values in a row superscripted by different letters are significantly different at p < 0.05.

AIA-Amylase inhibitory activity

VD- Vacuum Dried

Fresh miniature Ambarella exhibited a significantly higher ( $p < 0.05$ ) TPC ( $3.36 \pm 0.11$  mg; GAE/g) than fresh large Ambarella ( $3.05 \pm 0.00$  mg; GAE/g). Significant differences ( $p < 0.05$ ) were observed among fresh Ambarella and their VD powders for TPC. VD powder of large Ambarella exhibited significantly higher ( $p < 0.05$ ) TPC ( $1.49 \pm 0.11$  mg; GAE/g) than VD powder of miniature Ambarella ( $1.25 \pm 0.04$  mg; GAE/g).

TPC of Sri Lankan Ambarella studied by Silva and Sirasa, the TPC of methanolic extracts of fresh samples of Ambarella was  $33.5 \pm 1.3$  mg; GAE per 100g [16]. In the present study TPC of 100g of fresh large Ambarella fruit is around 305.0 mg; GAE and fresh miniature Ambarella fruit is around 336.0 mg; GAE. According to the present study TPC of the Sri Lankan Ambarella varieties tested had more TPC than the TPC of the Sri Lankan Ambarella studied by Silva and Sirasa, 2016 [16]. This might be due to the difference of maturity stage and/or the extraction methods used. The reduction of polyphenols and antioxidant compounds during thermal processing can be explained by thermal degradation of chemical structure and water solubility of those compounds [17-19].

**Comment [A4]:** I suggest correcting: Silva and Sirasa, 2016

### 3.1.2 TFC of ethanolic extracts of fresh Ambarella fruit and VD powders

TFC of ethanolic extracts of fresh Ambarella fruit and VD powders is given in Table 2. Results demonstrated no significant differences ( $p > 0.05$ ) observed in the two varieties of Ambarella extract for TFC between fresh and VD conditions. In present study fresh miniature Ambarella exhibited higher TFC ( $0.16 \pm 0.03$  mg; QE/g) than fresh large Ambarella ( $0.12 \pm 0.02$  mg; QE/g). Significant differences ( $p < 0.05$ ) were observed among fresh Ambarella and their VD powders for TFC. Flavonoids in aqueous solutions exhibited different sensitivity to heat treatment depending on their structure [18-19].

TFC of the Sri Lankan Ambarella studied by Silva and Sirasa, 2016 reported TFC of Ambarella was  $53.5 \pm 2.8$  mg; CE per 100g fresh fruit [16]. Those results cannot be compared with the present study since the TFC was expressed as Catechin equivalents.

### **3.1.3 FRAP of ethanolic extracts of fresh Ambarella fruit and VD powders**

FRAP of ethanolic extracts of fresh Ambarella fruit and VD powders are given in Table 2. Fresh large Ambarella exhibited significantly ( $p < 0.05$ ) higher FRAP ( $0.71 \pm 0.13$  mg; TE/g) than fresh miniature Ambarella ( $0.32 \pm 0.01$  mg; TE/g). VD powder of miniature Ambarella exhibited significantly higher ( $p < 0.05$ ) FRAP ( $4.19 \pm 0.06$  mg; TE/g) than VD powder of large Ambarella ( $1.64 \pm 0.19$  mg; TE/g).

According to the previous studies, FRAP of the Sri Lankan Ambarella studied by Silva and Sirasa, 2016 stated FRAP of Ambarella was  $11.28 \pm 0.40$   $\mu\text{mol FeSO}_4$  per g of fresh fruit [16]. Those values could not be compared with the results of the present study due to the differences of extraction method and the units used to express the results.

### **3.1.4 DPPH activity of ethanolic extracts of fresh Ambarella fruit and VD powders**

DPPH radical scavenging activity of fresh Ambarella fruit and VD powders is given in Table 2.

Fresh large Ambarella exhibited significantly higher ( $p < 0.05$ ) DPPH radical scavenging activity ( $3.57 \pm 0.31$  mg; TE/g) than fresh miniature Ambarella ( $2.13 \pm 0.23$  mg; TE/g). Results demonstrated no significant difference ( $p > 0.05$ ) among fresh large Ambarella ( $3.57 \pm 0.31$  mg; TE/g) and its VD powder ( $3.00 \pm 0.49$  mg; TE/g) for DPPH radical scavenging activity. However there was a significant difference among fresh miniature Ambarella ( $2.13 \pm 0.23$  mg; TE/g) and its VD powder ( $1.09 \pm 0.04$  mg; TE/g). A significant difference ( $p < 0.05$ ) was observed among the VD powders of two varieties of Ambarella for DPPH radical scavenging activity.

The sample extracts were studied for dose response relationship and results were expressed as  $IC_{50}$ . A significant difference ( $p < 0.05$ ) was observed between the two varieties of Ambarella extract for DPPH radical scavenging activity with  $IC_{50}$  value.

### **3.1.5 ABTS radical scavenging activity of ethanolic extracts of fresh Ambarella fruit and VD powders**

ABTS radical scavenging activity of fresh Ambarella fruit and VD powders is given in Table 2.

Results demonstrated no significant differences ( $p > 0.05$ ) between the two varieties of fresh Ambarella extract for ABTS radical scavenging activity. Further results exhibited no significant difference ( $p > 0.05$ ) among fresh large Ambarella ( $3.83 \pm 0.16$  mg; TE/g) and its VD powder ( $4.03 \pm 0.27$  mg; TE/g). However there was a significant difference among fresh miniature Ambarella ( $3.87 \pm 0.15$  mg; TE/g) and its VD powder ( $3.06 \pm 0.09$  mg; TE/g). VD powder of large Ambarella exhibited significantly higher ( $p < 0.05$ ) ABTS radical scavenging activity ( $4.03 \pm 0.24$  mg; TE/g) than VD powder of miniature Ambarella ( $3.06 \pm 0.09$  mg; TE/g).

The sample extracts were studied for dose response relationship and results were expressed as  $IC_{50}$ . Fresh large Ambarella exhibited significantly higher ( $p < 0.05$ ) ABTS radical scavenging activity ( $IC_{50}$ :  $599.9 \pm 24.8$   $\mu$ g/ml) than fresh miniature Ambarella ( $IC_{50}$ :  $664.7 \pm 27.3$   $\mu$ g/ml). Further, significant differences ( $p < 0.05$ ) were observed among fresh Ambarella and their VD powders for ABTS radical scavenging activity with  $IC_{50}$  value.

### **3.1.6 Alpha-Amylase inhibition activity**

Alpha-Amylase inhibition activity of fresh Ambarella fruit and VD powders are given in Table 2. Results demonstrated no significant differences ( $p > 0.05$ ) between the two varieties of fresh Ambarella extracts for  $\alpha$ -Amylase inhibition activity. Significant differences ( $p < 0.05$ ) were observed among fresh Ambarella and their VD powders for  $\alpha$ -Amylase inhibition

activity. Results exhibited no significant differences ( $p > 0.05$ ) between the VD powders of two varieties of Ambarella for  $\alpha$ -Amylase inhibition activity. A study conducted by Joseph *et al.*, 2015 stated the hydro alcoholic Ambarella leaf extracts at a concentration 100  $\mu\text{g/ml}$  exhibited 69% of  $\alpha$ -amylase inhibitory activity showing the low  $\alpha$ -amylase inhibitory activity than its leaves [21].

Further, VD powders of Ambarella had a low  $\alpha$ -amylase inhibition activity than fresh Ambarella which means the heat treatment causes the reduction of  $\alpha$ -Amylase inhibition activity [22].

### **3.2 Formulation of convenient Ambarella incorporated soup mix**

In the present study, VD powder of large Ambarella was used for the product development activity because of its availability in the local market.

#### **3.2.1 Pre-processing of raw materials**

The development of VD Ambarella incorporated soup mix was selected for the study. The ingredients were chosen based on the following aspects. Corn flour and red rice flour were used as starch based thickening property. Green gram was used as an ingredient since it contains high amount of protein and fiber. The preparation of rice flour, corn flour and green gram flour were underwent preprocessing steps such as soaking, cooking in excess boiled water, drying and milling. Those preprocessing steps were important in preparation of ready to serve product to minimize the anti-nutrient compounds such as trypsin inhibitors in green grams [21].

#### **3.2.2 Determination of the best formula**

Three formulas of soup mix were developed (Table 1). Results of the mean rank scores were presented in Table 3. It was seen that the formula-2 had the highest mean rank scores for all sensory attributes except colour which was further shown in the radar chart (Figure 1).

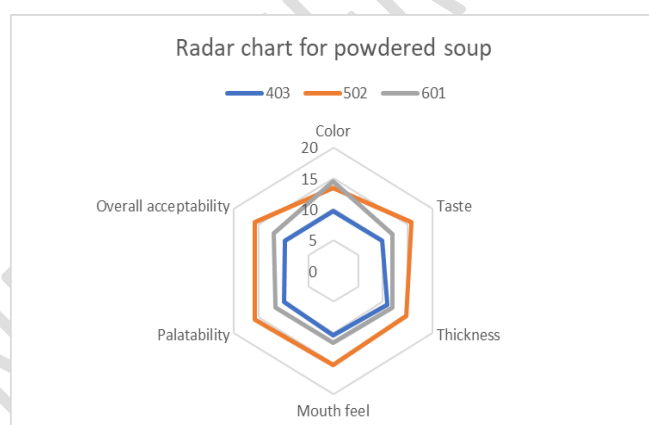
**Table 3: Mean rank scores for the convenient powdered soup mix**

Sample & code number	Colour	Taste	Thickness	Mouthfeel	Palatability	Overall acceptability
Formula -1 403	9.63	9.81	10.88	10.5	10.0	9.69
Formula -2 502	13.38	15.75	14.63	15.31	15.75	15.75
Formula -3 601	14.5	11.94	12	11.69	11.75	12.06

Formula -1 (403) –Soup with 15% of Ambarella powder

Formula -2 (502) –Soup with 20% of Ambarella powder

Formula -3 (601) –Soup with 25% of Ambarella powder



**Figure 1: Radar chart of variations of sensory attributes of the powdered soup samples.**

403- Formula-1

502- Formula-2

601- Formula-3

### 3.2.3 Evaluation of proximate composition and functional properties

The proximate composition of soup mix i.e. fat, fiber, ash, protein, carbohydrate and moisture contents were  $2.02 \pm 0.06\%$ ,  $4.49 \pm 0.07\%$ ,  $2.89 \pm 0.26\%$ ,  $11.59 \pm 0.02\%$ , 69.24 and  $9.77 \pm 0.14\%$  respectively. The average moisture content of powdered soup is less than 10% and it is an important factor for retaining microbial stability.

The nutritional composition analysis per serving size of 10g and functional properties of the best formula of selected soup mix are given in Table 4.

**Table 4: Proximate composition and functional properties of powdered soup mix**

Nutritional parameter	Nutrient value per serving size (10.0g)	Functional parameter	value
Energy (Kcal)	34.5	TPC (mg GAE/g)	0.55±0.00
Fat (g)	0.20	TFC (mg QE/g)	0.04±0.00
Crude fiber (g)	0.45	FRAP (mg TE/g)	0.035±0.15
Protein (g)	1.16	ABTS (mg TE/g)	330.08±11.58
		(IC <sub>50</sub> µg/ml)	1.65±0.06
Ash (g)	0.29	DPPH	ND
Carbohydrate (g)	6.92	α-AIA (% inhibition 100 µg/ml of extract)	39.49±0.29

Moisture % -  $9.77 \pm 0.14$

AIA -Amylase inhibitory activity

ND-Not Detected

#### 4. CONCLUSION

According to results obtained from the present study, fresh dwarf Ambarella showed the highest antioxidant potential in terms of TPC and TFC where as fresh large Ambarella exhibited high antioxidant potential in terms of FRAP and DPPH free radical scavenging activity.

Antioxidant potential of developed soup mix was less when compared with fresh fruit and VD powder. However, powdered soup mix exhibits higher α-amylase inhibitory activity than vacuum dried powder.

#### COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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