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PHYSICAL AND BIOCHEMICAL PROPERTIES OF AVERRHOA BILIMBI (BILIMBI).

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ABSTRACT

The project is aimed at analyzing the physicochemical and biological properties of Averrhoa Bilimbi powder with an ideal approach to exploit the underutilized properties of the fruit and to develop a functional food from it. The fruits were dried at temperatures 50˚, 60˚, and 70˚C. Efficient drying occurred at 70˚C with overall time of 240 minutes. An HPLC analysis of the dried powder was also conducted to determine the presence of any bioactive compounds, the properties of dried powder like pH, water activity, ash content, moisture content, carbohydrate content, protein content, ascorbic acid content, phenol content were analysed by AOAC methods. The fruits that were dried at 50˚C showed effective drying and good quercetin content. The pH value confirmed that nearly all the fruits were acidic. The ash content indicated that bilimbi fruits may be a potential source of many different bioactive compounds and organic acids. The fruits also were found to be a good source of carbohydrates, proteins etc. The amount of ascorbic acid, present in the fruit indicated that the fruits are a rich source of antioxidants. The phenolic content and oxalate content also determined the potential benefits of the fruit.

KEYWORDS: **Averrhoa bilimbi, drying, physical properties, chemical properties, quercetin.**

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INTRODUCTION

Averrhoa bilimbi (Fig. 1), that belongs to the Oxalidaceae family is an edible fruit that is underutilized. It is a native from South-East Asia and is cultivated in some parts of India, Malaysia, Indonesia, Singapore, Philippines, Thailand, Bangladesh and Myanmar.[1]. Traditionally, the leaves were used in variety of treatments like paste on itches, swelling, skin eruptions, cough, sites of poison etc. The decoction of fruits was also widely used for the treatment of inflammatory conditions.

Figure 1
***Averrhoabilimbi* fruits**

A. bilimbi grows in a small tree which grows up to 15 m with arranged branches. The leaves are hairy and forms clusters at the end of branches. The fruit juice is sour and extremely acidic. The plant is usually referred as cucumber tree in the English language. Alhassan *et al.,* stated that the juice of the fruit is used in the treatment of hyperbilirubinemia, cough and beriberi. Fruit syrup is considered as a remedy in pyrexia, inflammatory conditions and also in preventing the blood flow from rectum[2]. In an analysis conducted on Malaysia's *A. bilimbi* fruits, volatile constituents analysed were palmitic acid (20.4%), 2-furaldehyde (19.1%), and (Z)-9-octadecenoic acid (10.2%). In another study, Pino investigated the volatile constituents of *A. bilimbi* that is grown in Cuba. The fruit pulp showed approximately 6 mg/kg of total volatile components from 62 compounds. The major compounds were quercetin (2.7 mg/kg), Octane (0.29), tricosane (0.27 mg/kg), 2-furfural (0.18 mg/kg), and (Z)-9-tricosene (0.11 mg/kg). Lakshmi (2011) [3] reported that *A.bilimbi* fruit contains a large amount of oxalic acid. Excessive consumption of the fruit juice can lead to increased serum oxalate level and accumulation of calcium oxalate crystals. Nowadays, drying of food products are done to improve shelf life, reduce packaging costs, encapsulate original flavour and maintain nutritional value. The raw material that undergoes drying may change to a different material depending upon the drying mechanisms. No study has been done to determine the usage of dried powder to be used for medicinal purpose.Recent advancements in technology have played crucial roles in health care and medicinal plants have been scientifically evaluated mainly to find out more efficacious and safe biologically active agents responsible for various pharmacological activities of the medicinal plants. Hence the present work is aimed at investigating the physico-chemical and biological properties of *A. bilimbi*.

MATERIALS AND METHODS

Raw Material preparation

Bilimbi fruits of three different maturity stages were collected from Kerala. Chemicals were obtained from the Dept. of Food Process Engineering. The HPLC analyses were conducted at CARe KERALAM Ltd., KINFRA Small Industries Park, Kerala. The fruits were washed under running tap water and blow dried.

Physical properties of fruit

For measurement of the physical parameters the whole fruits were used whereas for all the other analysis the fruit powder was used. Analyses were conducted in triplicates. Length, breadth and thickness of approximately 10 fruit samples were measured using a vernier calliper. The average and standard deviation values were determined for all the three parameters. The maxima and minima of each parameter were identified.The geometric mean diameter (GMD) of the fruits were calculated by the formula given as,The unit volume was also determined based on the measured values of the 10 fruits using the formula given by Burubai (2007)[5] as The surface area was also determined using the formula given by Burubai(2007) [5] as The Sphericity was calculated using the method proposed by Burubai(2007)[5] as given below:The bulk density and true density of the fruits were measured using Davies(2009) [6]. The formula used to calculate the bulk density was-Where, M_b being mass of fruit and V_b the volume of container. True density was determined by,The porosity was estimated using Dash (2008) [7] as,The colorimetric readings of the samples were taken using Hunter colorimeter XD and the L, a, b and dE^* values were measured. The values were determined by placing the whole fruit as stated by Pranita (2014)[8]. 'L'

values denotes the lightness or darkness, 'a-'denotes the greenness while 'a+' is for redness. Similarly, 'b-'denotes blueness whereas 'b+' denotes yellowness of the fruit. The moisture content of the sample was determined using the standard AOAC (1990) [9] procedure. The moisture of the sample was lost by volatilization caused by heat. The formula used is as follows-The water activity was measured at room temperature using Water activity analyser (LAB Touch LT NOVASINA). If there is too much water in a product, there is a risk of microbial growth and water migration. Highly perishable foodstuffs have aw > 0.95. Growth of most bacteria is inhibited below aw of 0.91, yeasts below 0.87 and molds below 0.80. This can lead to clumping, changes in consistency and reduced shelf-life.

Drying Studies

Bilimbi fruits, obtained from Kerala were rinsed again in tap water and transversally cut (star-shaped) with a thickness of about 3 mm. To determine the drying curve, a laboratory scale static-tray dryer was used. Tuncay performed the experiments on bay leaves at air temperatures of 50, 60 and 70 °C at constant air velocity of 1m/s[10]. The dried samples were weighed using a precision balance. Weighing was made every 20 min. The drying process continued until the mass change between two weighing was less than 0.05 g. Drying experiments were repeated twice. Estimation of the bioactive compound quercetin present at various drying temperatures[10,11] was also done. In recent years, the drying behaviour of different products has been studied .These includes bay leaves, carrot, carambola etc.

Bio-chemical properties of bilimbi powder

All the chemical analysis was carried out using bilimbi powder. The pH of the samples was measured at 25±2 °C with a pH meter. The samples were mixed with an equal amount of SDW .Ascorbic acid was estimated using the Metaphosphoric acid- acetic acid method based on its ability to decolorize 2, 6-dichlorophenol-indophenol dye solution as devised by Kapur (2012)[12] . The formula used was-

$$\text{Standard AA conc. (mg/ml)} = 0.783 \times \text{(absorbance)}$$

$$\text{Ascorbic acid content} = \frac{\text{standard AA concentration} * \text{make up volume}}{\text{Volume of sample taken}}$$

The ash content was also determined based on the standard AOAC [9] procedure. The ash % was calculated by using the formula-

$$\text{Ash \%} = \frac{\text{Weight of ash} * 100}{\text{Weight of sample}}$$

Total phenol content of fruits of *A. bilimbi* extractives was measured employing the method proposed by Demiray[13]. The ethanol extract of bilimbi powder was used for analysis of total phenols. Total oxalic acid was determined according to Day and Underwood procedure (1982) [14]. Total carbohydrates and gross energy were calculated by the procedures outlined by Miller[15].

STATISTICAL ANALYSIS

In the present study, the results are expressed as mean and standard deviation (S.D.) of two determinations.

RESULTS AND DISCUSSIONS

Physical properties of fruit

The physical properties of fruits were measured and are given as in Table 1. The values of length (L), breadth (B), thickness (T1 and T2) were recorded. Average dimensions of the fruit were found out as length- 51.2 mm±13.86 and breadth-17.28 mm±5.0825. The maximum value of length is 71 mm and the minimum 31 mm. The maximum value of breadth is 29 mm and the minimum is 10.5 mm. The density of the fruit was found to be 1.03±0.15 g/cm^3.The porosity of the fruits that were found to 98.54±0.2368. All the volume, surface area, porosity data provides idea about the packing efficiency of the fruits.The water activity level was obtained as 0.9328± 0.0051 and it indicates that *bilimbi* fruits are moderately perishable. These values are in accordance with the findings of Narain (2001) [16].The value of moisture content 95.71±0.025 is in accordance with Narain (2001) [16] and hence plays an important role in determination of the shelf life of any products developed further from the fruits.The mature *bilimbi* fruits are greenish yellow in color with firm texture. This falls in accordance with Narain (2001)[16] and hence represents ripened stage of bilimbi.

Table 1
Physical properties of bilimbi fruit

Physical properties	Fruit
Length (mm)	51.2±13.86
Width (mm)	17.28± 5.0825
Thickness (mm)	17.444± 3.84057
Geometric mean diameter	24.897 ± 6.467
Volume (mm^3)	8076.779± 141.584
Surface Area (mm^2)	3291.29987± 699.0682
Sphericity (decimal)	0.486± 0.4666
a^w	0.9328± 0.0051
Bulk density (Kg/m^3)	10.03±0.15
True density (Kg/m^3)	70.218±0.023
Porosity (%)	78.54±0.2368
Galvanised steel (Coefficient of friction)	0.798±0.021
Aluminium (Coefficient of friction)	0.6168±0.022
Wood (Coefficient of friction)	0.238±0.0015
Moisture content(%)	95.71±0.025

Values are mean± SD

Drying studies

The drying temperature of 50 °C led to a total drying time of 470 min, while at 70 °C the drying time required was 240 min (Figure 2, Figure 3). It was thereby observed that the highest drying temperature neither altered the colour nor the texture under the experimental conditions tested. The drying of *bilimbi* in tray drier is possible at temperatures studied and the drying time is inversely proportional to temperature, as expected. The R^2 value was obtained as 0.951, 0.953, and 0.956

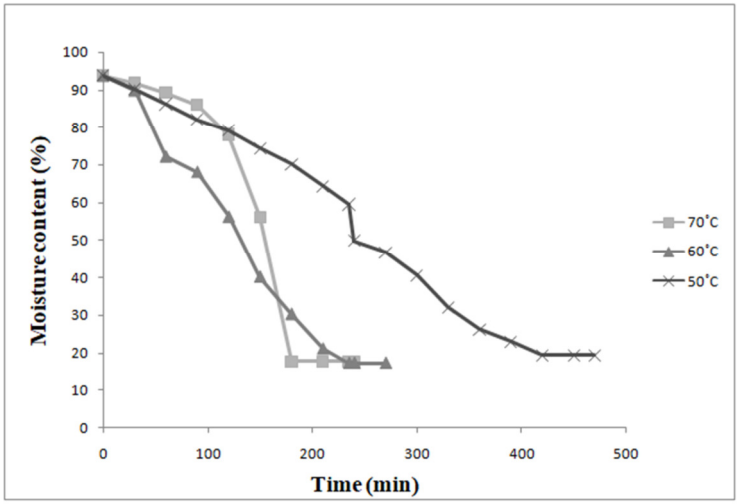

Figure 2
Drying kinetics of bilimbi-graph 1-Moisture content v/s Time graph

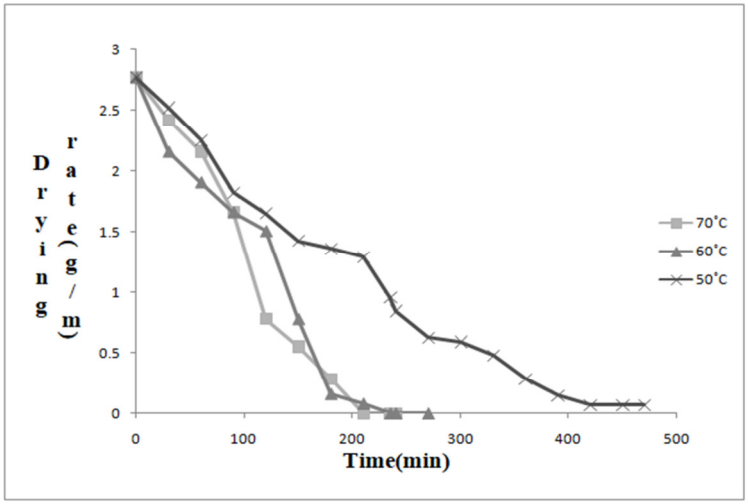

Figure 3
Drying kinetics of bilimbi-graph 2-Drying rate v/s Time graph

Estimation of quercetin content

Although efficient drying occurred at 70 ˚C with a drying time of 270 min, the bioactive compound quercetin is not retained in even small amounts (Figure 4,5,6) (Table 2). So the tray dried powder at 50 ˚C is taken as the standard and further analyses are carried out.

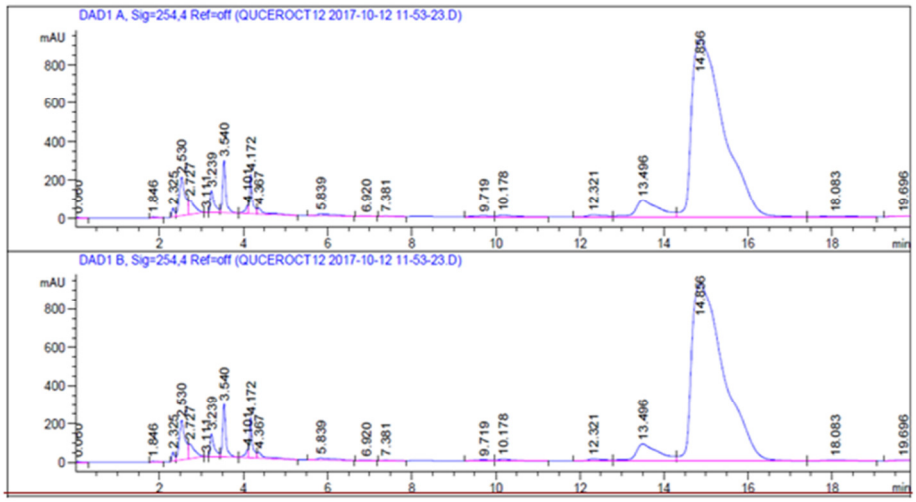

Figure 4
HPLC analysis of 50 ˚C powder

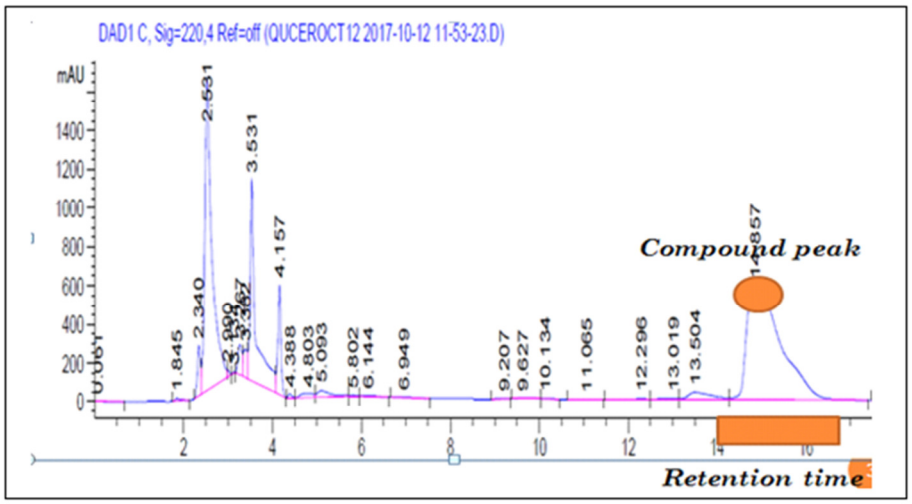

Figure 5
HPLC analysis of 60 ˚C powder

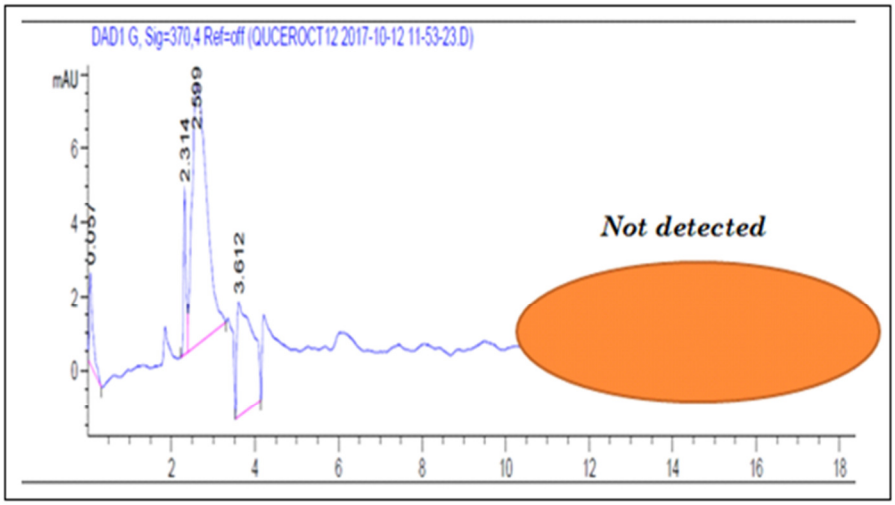

Figure 6
HPLC analysis of 70 ˚C powder

Table 2
Quercetin content at various drying temperatures

Temperature(˚C)	Amount of Quercetin(µg/ml)	Area of peak (%)	Retention time(min)
50	2.58	81.0051±16.285	14.915
60	0.6731	45.906±0.058	14.857
70	Not detected	Nil	nil

Bio-chemical properties

The bio-chemical properties of *bilimbi* powder is given in Table 3. The ash content in *bilimbi* was found out to be 4.68±0.058 % and the amount of organic matter was found out to be 95.32±0.256 %.These confirm that bilimbi is an excellent source of antioxidants and organic compounds[17].The pH of the fruit obtained was found out to be 1.665±.021213, which confirmed that the fruits are acidic.[18] Because of the acidic pH, the fruits undergo browning easily.Drying Studies to reduce the acidic content[18] of these fruits are hence helpful in wide acceptance of these fruits as a part of any functional food or supplement.The reducing sugar content was found out to be 2.76±0.59 g per 100 g and the required Recommended Dietary Allowances value being 3g/100g The ascorbic acid content of mature *bilimbi powder* was obtained as 15.833 ±.0598 mg/100 ml,which constitutes of about 25 % of RDA requirements and is in accordance with the amount of ascorbic acid present in *Averrhoa carambola* (15 mg/ml)[19], another species of Averrhoa as found by Anitha (2011) [2].The phenol content of the standardised *bilimbi* sample[20] is 58.24±4.28 mg of GAE/gof extract.The oxalic acid levels in bilimbi ranged between 8.01 and 9.6 mg/g. These high values of oxalic acid are probably responsible for the extremely low pH value of the fruit [21-22].

Table 3
Biochemical properties of bilimbi

Bio-Chemical properties	Values
Ash (%)	4.68 ± 0.058
pH	1.665±0.021213
Ascorbic Acid (mg/g)	15.833 ±.0598
Reducing sugar(g)	2.76±0.59
Protein (%)	6.238 ± 0.001
Phenol content(mg of GAE/g)	58.24±4.28
Oxalic acid(mg/g)	8.01-9.6

Values are mean± SD

ABBREVIATION

Degree	°
Celsius	C
High Performance Liquid Chromatography	HPLC
Association Of Official Analytical Chemists	AOAC
Percentage	%
Milligram	mg
Kilogram	kg
Geometric Mean Diameter	GMD
Surface area	A_s
Sphericity	S
Density	ρ
Mass	M
Volume	V
Square metre	mm^2
Cubic metre	mm^3
Porosity	ε
Millilitre	ml
Ascorbic Acid	AA
Microgram	µg
Millimetre	mm
Water activity	A^w
Minute	min
Milligram	mg
Gallic Acid Equivalents	GAE
Recommended Dietary Allowances	RDA

CONCLUSION

This study reveals that Averrhoa fruits are acidic, with high antioxidant, phenolic and carbohydrate content. Physical properties and dimensions of mature Averrhoa fruits prove that they are an ideal source for human consumption that was unexplored. The properties like sphericity and roundness helps in the designing of

suitable equipments for the large scale processing of the fruits. The mature bilimbi fruits are greenish yellow in color with firm texture.From the drying studies carried out, it was observed that the highest drying temperature could be used because neither the colour nor the texture was altered under the experimental conditions tested. Since drying of the fruits under the experimental conditions offers satisfactory results the fruits can be stored and preserved throughout the off- seson.In the HPLC analysis of the fruit, the bioactive component quercetin is detected and its amount in bilimbi is also determined. Although efficient drying occurred at 70 ˚C with a drying time of 270 min, the bioactive compound quercetin is not retained in even small amounts. Based on the studies carried out, the mature bilimbi powder obtained by tray drying at 50˚C being a rich source of antioxidant and polyphenol quercetin can be used for the development of a functional food.Since the ascorbic acid content, phenolic content and the oxalic content of the fruits satisfies the Recommended Dietary Allowances, the developed functional foods will not cause any harm to the consumer and inturn will help in regulating the overall well-being of an individual

AUTHOR CONTRIBUTION STATEMENT

Dr. K.A.Athmaselvi and S.Sasikala conceived of the presented idea. Athira J Subhash and Dr. K.A.Athmaselvi developed the work plan and performed the computations. K.A.Athmaselvi and S.Sasikala verified the analytical methods and encouraged Athira J Subhash to investigate on the amount of bioactive compound quercetin present in *Averrhoa bilimbi* and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

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