RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 41 Issue 1: 101-105 (March 2022)

Thermal Processing and Fermentation Improves the Physicochemical and Functional Properties of Dehydrated Agathi Leaves (*Sesbania grandiflora* L.)

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10.18805/ajdfr.DR-1602

ABSTRACT

Background: Green leafy vegetables (GLVs) are seasonal and perishable nature. It undergoes substantial-quality changes immediately after harvest that may lead to loss of its freshness. Hence this experiment was aimed to process and preserve Agathi leaves by dehydration and study the influences of thermal processing and fermentation on its physicochemical and functional properties.

Methods: Agathi leaves were separted from stalks, cleaned, washed then subjected to thermal treatments such as boiling (20 min), simmering (10 min) and steaming (10 min) followed fermentation with Baker's yeast (3% w/w concentration, 20 hours incubation at pH 6.5, 30°C temperature). The processed leaves were cabinet dried at 60°C for 4-5 hours then powdered and stored in an airtight container. The physicochemical and functional properties of the Agathi leaf powders were determined by following standard methods.

Result: The processed Agathi leaf powders contain 5.60-8.10% moisture, 35.12-45.90 g/100 g protein, 6.47-11.41 g/100 g minerals and 7.74-9.75 g/100 g crude fibre. Bulk density, Water absorption capacity, Oil absorption capacity, Emulsifying activity and Foaming capacity of the leaf powders were ranged from 0.56 to 0.67 g/mL, 2.69 to 3.95 g/g, 0.96 to 1.23 g/g, 48.26 to 67.73% and 23.06 to 51.83% respectively. Thermal and fermentation treatments have significantly changed/altered the physicochemical and functional properties of Agathi leaf powders. The findings suggest that Agahti leaf powder is rich in protein, minerals and crude fibre and showed good functional properties that could be used to develop functional food products.

Key words: Agathi leaf, Cabinet drying, Fermentation, Functional properties, Physicochemical, Thermal processing.

INTRODUCTION

Green Leafy Vegetables (GLVs) are commonly referred to as "poor man's vegetables" because they are the most reliable and affordable source of high-quality nutrients such as vitamins, minerals, dietary fiber *etc.* for underprivileged (Ifesan *et al*., 2014; Yadav *et al*., 2013). However, the average consumption of GLVs in India was 24 g/CU/day that well below the recommended level of 40 g/CU/day. It shows a large gap between actual and recommended GLVs consumption levels despite the decades of concern and publicity. In recent years, malnutrition especially micronutrient deficiencies became a major public health concern and there is a need to break the inter-generational cycle of malnutrition through diet-based approaches (Sreenivasa Rao, 2017).

The GLVs are a well-known source of micronutrients and consumed mostly in cooked form. Fresh GLVs are commonly preferred for cooking however, their seasonal nature and perishability due to its water content (70-92%) limits its usage in the food. After harvest, GLVs undergo substantial compositional changes that lead to loss of its quality, especially freshness. If GLVs lose more than 3 percent of their original fresh weight, they are rendered unsalable hence it should be processed immediately to preserve it (Jane Ambuko *et al*., 2017). Furthermore, lack of knowledge on post-harvest handling and practices and inadequate transport and storage facilities may lead to a qualitative and quantitative loss in leafy vegetables (Gupta *et al*., 2013). High post-harvest losses encountered in leafy vegetables (upwards of 50%) may attribute to various biological and environmental factors where storage temperature and relative humidity play a central role in its deterioration/spoilage (Jane Ambuko *et al*., 2017).

Drying is the oldest technology widely used to preserve vegetables, especially when they are abundantly available. It brings about a substantial reduction in weight and volume; resulting in less expense towards packaging, storage and transportation costs and also enables storability of the product under ambient condition (Gupta *et al*., 2013; Ibarz and Barbosa-Canovas, 2000). Joshi and Mehta (2010) also

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How to cite this article: Sahul Hameed, R. and Manju Priya, P. (2022). Thermal Processing and Fermentation Improves the Physicochemical and Functional Properties of Dehydrated Agathi Leaves (*Sesbania grandiflora* L.). Asian Journal of Dairy and Food Research. 41(1): 101-105. DOI: 10.18805/ajdfr.DR-1602.

Submitted: 12-11-2020 **Accepted:** 08-12-2021 **Online:** 17-01-2022

reported that oven-dried *Moringa* leaves are a concentrated source of many nutrients such as carbohydrates, proteins, minerals and crude fibre and noticed a manifold increase in the nutrient content of dried leaves. This show that drying improves the nutritional value of GLVs and widens its scope for value addition.

Agathi (*Sesbania grandiflora*) is an underutilized GLV rich in minerals, vitamins, antioxidants and various phytochemicals. They also possess anxiolytic, anticonvulsive, hepatoprotective, antibacterial, anthelminthic, antitumor and contraceptive properties (Samira *et al*., 2017), helps in treating many diseases such as dysentery, stomatitis, fever, smallpox, sore throat, cancer, diabetes, headache, *etc.* (Bhat and Daihan, 2014, Nwozo *et al*., 2015). Researchers explored the medicinal value of Agathi leaves for the prevention and management of diseases/disorders in fresh leaves however the effect of processing on the nutritional, functional and biological activity of the Agathi leaves are least studied so far. Hence the study was envisaged to process/preserve Agathi leaves by drying/dehydration and determine the changes in their quality.

MATERIALS AND METHODS

Agathi leaves were collected from a local farm in Dindigul district, Tamil Nadu, India. The experts in the Department of Biology, School of Sciences, The Gandhigram Rural Institute - Deemed to be University, Gandhigram has verified plant species as Agathi (*Sesbania grandiflora*). Baker's dry yeast (*Saccharomyces cerevisiae*) and Potassium Meta-bisulfite (KMS) were purchased from Supermarket in Dindigul. The chemicals used for analyzing the physicochemical properties, functional properties and phytochemicals in the leaf samples were analytical grade (AR). The study was conducted in the Department of Home Science, School of Sciences, The Gandhigram Rural Institute - Deemed to be University, Gandhigram, Tamil Nadu, India in the year 2019-2020.

Agathi leaves were separated from the stalks manually and checked for its quality. After removing defective/damaged sections, the leaves were washed twice with running tap water and surface dried. Then the leaves were loaded into a cabinet drier (Biotronic Instruments, India) pre-set to 60°C temperature and kept it for 4-5 hours. Dehydrated leaves were pulverized and packed in an airtight container. Then they were labelled as a control (T_0) and stored in the refrigerator until further analysis. To study the effect of thermal processing, fresh cleaned Agathi leaves were subjected to various heat treatments such as boiling (T_1), Simmering (T_2) and Steaming (T_3) for 10 minutes using distilled water followed by cabinet drying at 60°C for 4-5 hours.

The leaf powders were fermented with Baker's yeast (*Saccharomyces cerevisiae*) as follows: both control (T_0) and thermally processed (T_1, T_2 and T_3) Agathi leaf powders were dissolved with distilled water to a final concentration of 10% w/v and kept in a water bath temperature maintained at 30°C and shaking speed of 100 rpm for 30 minutes. After that, the suspensions were heated at 80°C for 10 minutes and added 0.1% w/w of Potassium Meta-bisulfite (KMS) and 3% w/w Baker's yeast powders to initiate the fermentation process. The suspensions were incubated in a shaking water bath (30°C temperature and 100 rpm speed) for 20 hours then cabinet dried at 60°C for 3-4 hours. After drying, they were powdered, packed in airtight containers separately and stored in a refrigerator.

The pH, proximate compositions of the leaf powders were analyzed as per the methods of AOAC (2000). Moisture content by using Digital Moisture Balance (Shimadzu, Japan), crude protein content by the Microkjeldhal method using Kelplus Nitrogen Analyzer (Pelican Equipments, Tamil Nadu, India), mineral content by muffle furnace,crude fibre contents by using the Fibroplus instrument (Pelican Equipments, Tamil Nadu, India) were determined and the results were expressed as g/ 100 g on a wet basis.

Functional properties such as bulk density (Kaur and Singh, 2005), water absorption capacity and oil absorption capacity (Lin *et al*., 1974), emulsifying activity and foaming capacity (Yasumatsu *et al*., 1972) of the leaf powders were determined by adopting standard protocols. The obtained results were expressed in g/mL for bulk density, g/g for water/oil absorption capacity, the percentage for both emulsifying activity and foaming capacity.

For analyzing phytochemicals in leaf powders, the aqueous extract was prepared as outlined by Karthikeyan and Vidhya (2019). Aliquot of extracts were tested for phenolic compounds, alkaloids, glycosides, steroids, tannin, saponin, flavonoids, quinone and coumarins adopting the methods described by Karthikeyan and Vidhya (2019) and Hasan *et al*. (2018).

All the analyses were carried out in triplicates. Data were pooled and analyzed by ANOVA using statistical package for social sciences (SPSS) software version 17. Duncan Multiple Range Test (DMRT) was used to differentiate means with significance at a 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

The physicochemical properties of Agathi (*Sesbania grandiflora*) leaf powders are presented in Table 1. The yield of leaf powders was ranged between 14.5 and 23.9% irrespective of the treatments. Compared to the control (T_0-22.4%), the higher yield was noticed in steamed leaf powder (T_3-23.9%). A significant ($p<0.05$) reduction in the yield of thermally processed and fermented leaf powders may be due to leaching of soluble matters in the leaves to water during the thermal processing as well as utilization of nutrients in the leaves by the fermenting microorganism for their growth/multiplication respectively. The pH of control leaf powder was 5.89 (T_0), which was increased significantly ($p<0.05$) to 6.05-6.25 in thermally processed samples and 7.12-7.23 in fermented samples. The findings suggest that boiling caused severe damage to the plant cells/tissues and

released volatile acids in the leaves. As a result, the pH of boiled leaf powders might have been increased.

A significant difference ($p<0.05$) between the moisture content of control and processed Agathi leaf powders was observed. The moisture content of the leaf powders was reduced from 7.53 (T_0) to 6.70, 5.91 and 5.60% for boiled, simmered and steamed samples (T_1, T_2 and T_3) respectively. FT_0 showed slightly higher moisture content (8.10%) than the control. The values were comparable to the results reported by Gupta *et al*. (2013), who found that the moisture content of blanched and oven-dried GLVs was ranged between 3.5 and 7.9%.

Fermented leaf powder exhibited higher protein content of 44.23 g/100 g (FT_0) than the unfermented leaf powder (T_0 - 35.59 g/100 g). T_2 had a higher protein content of 38.88 g/100 g among thermally processed leaf powders and FT_1 recorded the highest protein content of 45.90 g/100 g in fermented leaf powders. It seems thermal processing and fermentation increased the protein content of leaf powders. Ifesan *et al*. (2014) also found that the protein content of some commonly consumed Nigerian GLVs was increased after oven drying at 50°C which ranged between 14.27 and 30.26 g/100 g. Researchers also postulated that high protein content observed in fermented leaf powders might be due to the biomass of yeast and bio-conversion of carbohydrates into microbial protein by intermediary metabolism, nitrogen-fixing ability, or microbial growth (Jannathulla *et al*., 2017; Cui *et al*., 2012).

The mineral content of control Agathi leaf powder (T_0 - 9.06 g/100 g) was significantly lower ($p<0.05$) than the mineral content of fermented control leaf powder (FT_0 - 10.319.06 g/100 g). It seems boiling and steaming treatments have influenced the mineral content in the leaf powders (11.41 (T_1) and 9.71 g/100 g (T_3) than simmering (T_2 8.71 g/100 g). Similar trends were observed in fermented leaf powders. The results suggest that thermal and fermentation treatments increased the leaf powders' mineral content due to breaks down of the complexes between minerals and other biomolecules. Acho and coworkers (2014) also reported high mineral content in dried leafy vegetables compared to fresh leaves, ranging from 8.53 to 22.20 per cent. While boiling of the leaves for a longer duration (15, 30 and 45 min) before drying has reduced the mineral content by 12.42-54.09%. Hence, the leafy vegetables should be boiled for less than 15 min to avoid leaching minerals from the leaves into the water.

Table 1: Physicochemical properties of Agathi (*Sesbania grandiflora*) leaf powders.

Treatments	Physicochemical properties of Agathi leaf powder					
	Yield (%)	pH	Moisture (%)	Protein (g/100 g)	Minerals (g/100 g)	Crude fibre (g/100 g)
T_0	22.44±0.21^{b}	5.89±0.02^{e}	7.53±0.33^{b,a}	35.59±0.91^{e,f}	9.06±0.04^{d}	8.57±0.01^{b}
T_1	18.18±0.17^{c,d}	6.25±0.04^{c}	6.70±0.01^{b}	35.12±0.46^{f}	11.41±0.14^{a}	9.75±0.14^{a}
T_2	20.55±0.19^{c}	6.05±0.01^{d}	5.91±0.79^{c}	38.86±0.40^{d}	8.71±0.75^{d}	8.90±0.57^{b}
T_3	23.91±0.30^{a}	6.15±0.03^{c}	5.60±0.44^{c}	37.02±1.25^{e}	9.71±0.24^{c}	8.84±0.29^{b}
FT_0	17.95±0.09^{d}	7.12±0.07^{b}	8.10±0.25^{a}	44.23±1.01^{b}	10.31±0.19^{b}	8.48±0.02^{b,c}
FT_1	14.54±0.11^{e}	7.21±0.06^{a}	7.18±0.09^{b}	45.90±0.49^{a}	9.30±0.20^{c,d}	9.49±0.22^{a}
FT_2	16.44±0.21^{d}	7.23±0.03^{a}	6.86±0.33^{b}	41.09±1.47^{c}	6.47±0.15^{e}	8.11±0.16^{c,d}
FT_3	19.13±0.37^{c}	7.18±0.25^{b}	7.97±0.30^{a}	40.47±0.65^{c}	9.13±0.30^{d}	7.74±0.18^{d}

Values with different superscripts in the same column were significantly different ($p<0.05$).

T_0, T_1, T_2 and T_3 denote control, boiled, simmered and steamed leaf powder respectively.

FT_0, FT_1, FT_2 and FT_3 represent fermented T_0, T_1, T_2 and T_3 leaf powder respectively.

Table 2: Functional properties of Agathi (*Sesbania grandiflora*) leaf powders.

Treatments	Functional properties of Agathi leaf powder				
	Bulk density (g/mL)	Water absroption capacity (g/g)	Oil absroption capacity (g/g)	Emulsifying activity (%)	Foaming capacity (%)
T_0	0.59±0.005^{c}	2.69±0.010^{e}	1.01±0.145^{b,a}	48.27±6.740^{c}	38.92±1.944^{c}
T_1	0.60±0.011^{b,c}	3.49±0.030^{b}	1.12±0.070^{a}	52.21±0.975^{c}	23.06±0.005^{e}
T_2	0.67±0.005^{a}	3.32±0.110^{b,c}	1.12±0.085^{a}	54.50±3.040^{b,c}	25.63±0.889^{e}
T_3	0.57±0.000^{d}	3.11±0.195^{c,d}	0.96±0.120^{b}	48.32±3.780^{c}	40.69±0.819^{c}
FT_0	0.59±0.004^{c}	3.94±0.095^{a}	1.23±0.050^{a}	67.73±2.690^{a}	40.52±0.55^{c}
F_1	0.62±0.004^{b}	2.70±0.120^{e}	1.20±0.050^{a}	58.45±4.700^{b}	30.99±0.873^{d}
F_2	0.55±0.000^{e}	3.06±0.086^{d}	1.20±0.030^{a}	51.26±5.550^{c}	47.86±1.913^{b}
FT_3	0.62±0.008^{b}	3.29±0.125^{c}	0.96±0.110^{b}	58.89±0.395^{b}	51.83±4.907^{a}

Values with different superscripts in the same column were significantly different ($p<0.05$).

T_0, T_1, T_2 and T_3 denote control, boiled, simmered and steamed leaf powder respectively.

FT_0, FT_1 FT_2, FT_3 represent fermented T_0, T_1, T_2 and T_3 leaf powder respectively.

Table 3: Phytochemicals in Agathi (*Sesbania grandiflora*) leaf powders.

Phytochemicals	Treatments							
	T_0	T_1	T_2	T_3	FT_0	FT_1	FT_2	FT_3
Phenol	+++	++	+	+++	++	-	+	++
Steroid	++	+	+	++	+++	+	+	+
Alkaloid	+	++	+++	+	++	-	-	++
Glycoside	++	+	+	+	+++	+	++	+++
Saponin	+++	+	++	+	++	+	++	+
Tannin	++	-	-	+	+	-	-	+
Quinone	+++	+	-	++	+++	++	++	+++
Flavonoid	+++	+	+	+	+++	+++	+++	+++
Anthocyanin	-	-	-	-	-	-	-	-
Coumarin	+++	+++	+++	+++	+++	+++	+++	+++

"+, ++ and +++" Indicates the high, moderate and low presence of the particular constituents respectively, "-" Indicate the absence of the particular constituents; T_0, T_1, T_2 and T_3 denotes control, boiled, simmered and steamed leaf powder respectively; FT_0, FT_1, FT_2, FT_3 represents Fermented T_0, T_1, T_2 and T_3 leaf powder respectively.

The crude fibre content of control and treated Agathi leaf powders were ranging from 7.74 to 9.75 g/100 g. Within the treatments, T_1 and FT_1 recorded higher crude fibre content of 9.75 and 9.49 g/100 g respectively which was followed by T_2 and FT_2 (8.90 and 8.11 g/100 g respectively) and T_3 and FT_3 (8.84 and 7.74 g/100 g respectively). The significant difference ($p<0.05$) observed in the crude fibre content of boiled leaf powder with others was probably due to loss/leaching of soluble matters in the water during boiling, resulting in the concentration of crude fibre. Ifesan *et al*. (2014) also found a difference in crude fibre content of unfermented (12.90%) and fermented *T. occidentalis* leaves (12.67%).

Functional properties of thermally treated and fermented Agathi (*Sesbania grandiflora*) leaf powders are given in Table 2. The bulk density of the control leaf powder was 0.59 g/mL (T_0) and it differs significantly ($p<0.05$) with thermally processed leaf powders (0.57-0.67 g/mL) as well as fermented leaf powders (0.56-0.63 g/mL). Kaur *et al*. (2008) reported that the bulk density of the cabinet dried mustard, mint and spinach leaves were lower than the sun-dried leaves which varied from 55.1 to 171.5 kg/m^3.

WAC is an index to measure the maximum amount of water that a food product would absorb. It was significantly higher ($p<0.05$) for both thermally processed and fermented Agathi leaf powders (2.79-3.94 g/g) than the control (2.69 g/g). FT_0 recorded the highest WAC of 3.95 g/g followed by T_1 (3.49 g/g) and T_2 (3.32 g/g) leaf powders which may due to increased concentration of carbohydrate, protein and crude fibre in the leaf powders. OAC of control leaf powder was comparable to processed leaf powders (1.02-1.23 g/g) and it showed the least changes in OAC with the different treatments except for FT_3 (0.96 g/g). Ijarotimi and coworkers (2013) also found high WAC in fermented Moringa seed flour (140 g/mL) than that of raw seed flour (80 g/mL).

The emulsifying activity of control Agathi leaf powder was 48.27% (T_0) whereas it was increased significantly ($p<0.05$) to 52.21 and 54.50% for boiled (T_1) and simmered (T_2) leaf powders respectively. Compared to other treatments, FT_0 showed much higher emulsifying activity ranged from 51.26 to 67.73%. The foaming capacity of fermented Agathi leaf powders was much better than the thermally processed leaf powders. High foaming capacity was noted in FT_3 (51.83%) and low in T_1 (23.06%). These findings suggest that emulsion activity and foaming capacity were better in fermented leaf powders than others which could be attributed to its high protein content and having the ability to stabilize foam and emulsion structure.

The aqueous extract of control leaf powder showed the presence of phytochemicals like phenols, steroids, alkaloids, glycosides, tannins, quinones, flavonoids and coumarins and absence of anthocyanin. This suggests that the phytochemicals in Agathi leaf powders were soluble in water (Table 3). There were noticeable changes in the phytochemicals of thermally processed and fermented leaf powders, whereas steaming with and without fermentation exhibited a better profile than boiling and simmering treatments. Gupta and Apte (2018) reported there was a significant ($p<0.05$) difference in the yield of aqueous and ethanolic extracts prepared from *Sesbania grandiflora* leaves which were 25.8 and 16.8 percent respectively. Phenolic compounds, flavonoids and saponins were significantly higher ($p<0.05$) in ethanolic extract of the leaves compared to aqueous extract, whereas tannins were higher in aqueous extract of the leaves. The authors found that *Sesbania grandiflora* leaves had good antioxidant and cytotoxicity that could be used to develop drugs against various human diseases.

CONCLUSION

From the results, it is evident that Agathi leaf powder contain a good amount of protein (30-45%), minerals (6.5-11.5%) and crude fibre (7.75-11.75%). It also showed good water/oil absorption capacities and moderate emulsifying and

foaming capacities. Phytochemical analysis revealed many of the phytochemicals in Agathi were soluble in water. There was a change in the physicochemical/functional properties and phytochemicals profile of leaf powders due to thermal processing and yeast fermentation. The findings suggest Agathi leaf powder has the potentials to develop nutritious and functional food products.

ACKNOWLEDGEMENT

The authors would like to thank Ms. Nithya, Lab Technician and Ms.I. Maheswari, Ph.D. Scholar, Ms. J. Kowsalya, II M.Sc. Food Science and Nutrition, Ms. Musfirabanu, Ms. Nandhini, Ms. Nirmala and Ms. Yuvathi, III year B.Sc. Home Science students, Department of Home Science, The Gandhigram Rural Institute-Deemed to be University, Gandhigram for their timely help in completing the analytical work.

Conflict of Interests

The authors declare no conflict of interest.

Authors' contributions

Dr. R. Sahul Hameed has designed the study and prepared the manuscript. Ms. P. Majupriya has conducted the laboratory experiments, done a statistical analysis and reported the results.

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