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Full Length Research Article

Formulation, Standardization, and Quality Evaluation of *Sesbania grandiflora* Incorporated Soup Cubes for Astronauts

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Abstract

The number of Indian astronauts visiting space for missions will increase in the coming years. The primary goal of this research collaborating with Vaayusastra aerospace (IIM incubated company) is to develop a space food for Indian astronauts. The *Sesbania grandiflora*-incorporated soup cubes were created by combining *Sesbania grandiflora* leaves with other vegetables and herbs in ratios of 70:30 (T_1), 60:40 (T_2), and 50:50 (T_3). The soup cubes were freeze-dried at -80 degrees Celsius for 30 hours and the moisture content of the soup cube was found to be 0.36%. Sensory evaluation was performed with 20 untrained panelists using a 9-point hedonic scale. Among treatments T_1, T_2, and T_3, T_3 was accepted overall acceptability of 8.7 ± 0.47. According to the proximate analysis results, the treatment T_1 (70:30) was superior to the other treatments in terms of energy, protein (164mg), CHO (64.1mg), fat (5.8g), calcium (110mg), and iron (9.34g). The total bacteria count was 2.09 × 104 CFU/ml, which is negligible, and the total yeast mold count was nil, according to the microbial analysis. The product was microbially safe for human consumption as a ready-to-cook space food. As a result, the soup cube is an excellent ready-to-cook space food for astronauts.

***Key words*:** *Sesbania grandiflora*, Sensory analysis, Conventional soup cubes, Space food, Calcium-rich soup cubes

Various plant parts, including leaves, stems, flowers, fruits, and roots, are consumed from the diverse group of vegetables, which are primarily annual plants. They are nutrient-rich and crucial parts of a diet that is well-balanced. A nutrient-rich food category of vegetables is green leafy vegetables. The herb *Sesbania grandiflora* leaves, also known as omnipotent spinach, has the ability to treat both physical and mental conditions in people. *Sesbania grandiflora* is its botanical name, and the Siddha school of medicine lists exactly 63 therapeutic advantages. This reduces body temperature, mends injuries, and works well to treat illnesses like mental depression (Arfan *et al*. 2016).

The main requirement for astronauts during space flight is calcium. This will be due to the effect of microgravity on human body (Gabel *et al*., 2022). The main objective of this study is to formulate a food product concerning calcium loss. The *Sesbania grandiflora* leaves incorporated soup cubes are rich in calcium to meet the astronaut's calcium requirement (Chaloulakou *et al*. 2022).

Following that, Ramesh *et al*. reported that *Sesbania grandiflora* aqueous suspension significantly reduced elevated hepatic, renal, and lipid peroxidation markers, improved antioxidant levels, and restored hepatic and renal architecture in cigarette smoke-exposed rats. Semwal et al. reported a significant neuroprotective effect in celecoxib-treated mice via cholinergic system modification or oxidative stress blockage and AchE enzyme inhibition at 200 and 400 mg/kg doses in mice. *Sesbania grandiflora* leaves' antioxidant capacity protects the lung from oxidative damage (Mohiuddin 2019).

Fig 1 *Sesbania grandiflora* leaves

MATERIALS AND METHODS

Pre-processing of raw materials

Preparation of Sesbania grandiflora leaves soup puree

70g of *Sesbania grandiflora* leaves were selected and washed thoroughly to remove the dirt and impurities. 20g of onion, carrots and potatoes each were also selected and washed

thoroughly and peeled. The cleaned raw ingredients were chopped into small pieces and were cooked in open pan method with 5ml of oil, 50ml of water, 50g of salt, 5g of pepper and 50g of corn flour for 15 minutes at 120°C until they become soft. The cooked mixture was made into a puree with the help of a food processor (Dilrukshi and Senarath 2021).

Fig 2 Soup puree

Formulation and standardization of the soup

Sesbania grandiflora leaves soup cube was prepared by Saikia *et al*. (2015) with slight modifications. The pureed soup mixture was transferred into a pan and sautéed for 20 minutes until they have transformed into a semi-solid mixture. The puree was then analyzed by nine-point hedonic scale.

Sensory analysis for the soup before lyophilization

The sensory analysis of the soup before lyophilization was done by 25 semi-trained panelists using nine-point hedonic scale. The treatments T_1, T_2, T_3, and T_4 were analyzed and found to have similar desirability, out of which the treatment T_1 has the highest overall acceptability. The accepted treatment T_1 were given for lyophilization and other analysis (Valentina 2016).

Table 1 Proportion of ingredients used for various treatments

Sample	*Sesbania grandiflora* leaves	Onion	Carrot	Potato	Salt	Oil	Pepper	Corn flour
T_1	70g	20g	20g	20g	50g	5ml	5g	50g
T_2	60g	20g	20g	20g	50g	5ml	5g	50g
T_3	50g	20g	20g	20g	50g	5ml	5g	50g
T_4	40g	20g	20g	20g	50g	5ml	5g	50g

Lyophilization

The freeze-drying lyophilization will be performed with minor modifications to the procedure described by Saikia *et al*. (2015). The freeze dryer was cleansed thoroughly with cotton and ethanol. The semi-solid sample was filled in a silicone mold for acquiring a proper shape and covered with aluminum foil and small holes were perforated. These were then kept at average refrigerator temperature overnight and then kept in the tray freeze dryer for 20 hours at -80°C. The freeze-dried soup cubes were packed in mylar bags. The required samples were given for further analysis procedures (Ceballos *et al*. 2012).

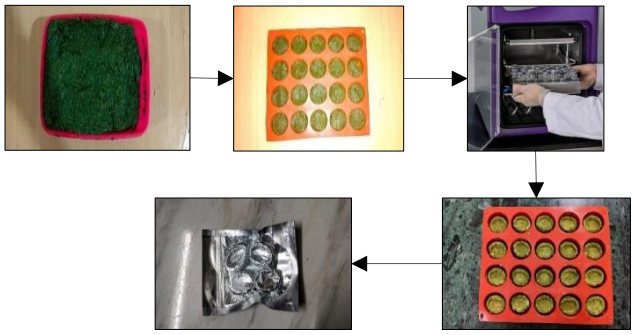

Fig 3 Lyophilization

Table 2 Rehydration ratio

Treatment	Quantity of water (100°C) (ml)	Soup cube (g)
T_5	75	4
T_6	100	4
T_7	125	4
T_8	150	4

Rehydration ratio

Rehydration characteristics are commonly used as a quality indicator for dried foods.

The rehydration properties, rehydration rate, and rehydration capacity were important characteristics related to their later preparation for consumption (Ansari *et al*. 2020).

Sensory analysis of the soup cube to determine the best rehydration level

Sensory analysis examines the properties (texture, flavor, taste, appearance, smell, and so on) of a product or food using the panelists' senses (sight, smell, taste, touch, and hearing). For centuries, this type of analysis has been used to accept or reject food products. Historically, it was regarded as a methodology for assessing food quality that supplemented technological and microbiological safety (Chaudhari and Solanke 2013).

The overall acceptability of the soup with 4 variations has been found through 25 semi-trained panelists using nine points hedonic scale. T_7 variation with 125ml of water was accepted from the results.

Physicochemical analysis

Physical analysis of the soup

The formulated *Sesbania grandiflora* leaves soup cube was analyzed for physical properties such pH, rehydration ratio, moisture content, and viscosity. The line spread test will evaluate the soup's consistency. The most important reason for monitoring pH is food safety, but pH also influences the quality of food products. Broths and soups are usually water-based and have the potential to be very hydrating and nutritious (Ansari *et al*. 2021).

Rehydration characteristics are commonly used as a quality indicator for dried foods.

Many products' rehydration properties, rehydration rate, and rehydration capacity were important characteristics related to their later preparation for consumption (Mitra and Sharma 2020).

Proximate composition

The Proximate composition of the formulated *Sesbania grandiflora* leaves soup cube was evaluated. Food proximate composition includes their iron, calcium, vitamin A, protein, and carbohydrate contents. The food industry may be interested in these food components for product development, quality control (QC), or regulatory purposes (Farzana *et al*. 2017).

Microbial analysis

Microbial analysis was performed using the total plate count method. The total plate count was determined by examining the colonies that formed, specifically bacteria

(Premakumar *et al*. 2018). The maximum bacterial load allowed for beverages is $1.0x10^4$ CFU/ml, according to (Rashed *et al*. 2013, Doddola *et al*. 2008).

Table 3 Microbial analysis

Microbes	Methods
Total bacterial count	As per AOAC, 2000
Total yeast and mold count	As per AOAC, 2000

Shelf-life analysis

The soup cubes were wrapped with aluminum foil and packed in mylar bags, and stored at room temperature to assess the microbial content in the soup cubes which is to find out the total bacteria, yeast, and mold count (Robert Koch 1881). The assessment of microbial content will be done on the 1st day, 15th day, 30th, and 45th day (Singh and Kaur 2020).

RESULTS AND DISCUSSION

Sensory analysis for the soup before lyophilisation

The sensory analysis for the soup before lyophilization was initially tested for various treatments of ingredients to determine the treatment T_1 that was found to be the overall accepted treatment with the values 8.78±0.52.

Table 4 Sensory analysis for the soup before lyophilisation

Treatment	Colour	Flavour	Texture	Appearance	Taste	Overall acceptability
T_1	8.8±0.41	8.75±0.47	8.65±0.49	8.8±0.41	8.65±0.49	8.78 ± 0.52
T_2	8.75±0.44	8.7±0.47	8.65±0.49	8.45±0.60	8.45±0.60	8.5 ± 0.51
T_3	8.7±0.47	8.55±0.51	8.6±0.50	8.5±0.61	8.45±0.51	8.4 ± 0.50
T_4	8.8±0.41	8.5±0.51	8.6±0.50	8.45±0.60	8.35±0.67	8.75 ± 0.51

Sensory analysis of the soup cube

Sensory evaluation is the most important method for determining how effectively a food is obtained. Sensory evaluation of food products can be used to successfully create new products, improve existing products, and regulate quality. The main flavour and aroma of the ingredients should be reflected in the desired qualities of the soup. A product should be free of unpleasant flavours, tastes, objectionable aromas, and uneven textures. The rehydration ratio estimation showed that the treatment T_7 with the value 8.78±0.52 was discovered to be the most preferred in terms of colour, flavour, taste, appearance, texture and overall acceptability after being reconstituted in hot water (4g/125mL) (Ouattara *et al*. 2011).

Table 5 Sensory analysis for the soup cube

Parameters	Product type	Original order of mean	Product type	Ranked order of mean	Lsd value	P (at alpha)	Mean square error	No. of judges
Colour	T_5	8.80	T_8	8.60	0.128	0.05	0.264	20
	T_6	8.75	T_7	8.70				
	T_7	8.70	T_6	8.75				
	T_8	8.60	T_5	8.80				
Flavour	T_5	8.75	T_8	8.50	1.000	0.05	0.239	20
	T_6	8.70	T_5	8.55				
	T_7	8.55	T_6	8.70				
	T_8	8.50	T_7	8.75				
Texture	T_5	8.65	T_5	8.60	0.269	0.05	0.323	20
	T_6	8.60	T_6	8.65				
	T_7	8.65	T_7	8.65				
	T_8	8.70	T_8	8.70				
Appearance	T_5	8.80	T_5	8.45	0.454	0.05	0.397	20
	T_6	8.45	T_6	8.50				
	T_7	8.50	T_8	8.70				
	T_8	8.70	T_7	8.80				
Taste	T_5	8.65	T_5	8.45	0.414	0.05	0.333	20
	T_6	8.45	T_6	8.45				
	T_7	8.45	T_7	8.65				
	T_8	8.70	T_8	8.70				
Overall acceptability	T_5	8.70	T_5	8.40	0.225	0.05	0.267	20
	T_6	8.50	T_6	8.50				
	T_7	8.40	T_7	8.60				
	T_8	8.60	T_8	8.70				

Table 6 Physicochemical analysis

Composition	Treatment (T_1)
Moisture content	0.36
pH	6.3

Physicochemical analysis

Moisture content of the *Sesbania grandiflora* incorporated soup cube was found to be 0.36% which is subsequently lower than that reported by Omah *et al*. The pH of the soup cube was found to be 6.3 as in relevance with the findings of the study by Singh *et al*. (2018), Jhansi *et al*. (2021).

Proximate analysis

The proximate analysis of the *Sesbania grandiflora* leaves incorporated soup cube were shown in the (Table 7). The energy content present in the freshly prepared soup cubes were found to be 352 kcal estimated from the analysis. The vitamin A content estimated from the proximate analysis have showed the presence of 70.7 IU in the soup cube. The prepared sample

of *Sesbania grandiflora* incorporated soup cube have reliable values of fat content(g), protein(g), carbohydrates(kcal), calcium(mg) and iron(g) with contents 0.198, 16.4, 6.4, 550 and 9.3 respectively. The proximate values were similar to that resulted from thoodhuvalai (Solanum procumbens) leaves by Chandramouli *et al*. 2012, Rohmah *et al*. 2020.

Table 7 Proximate analysis

Composition	T_7
Moisture (%)	0.35±0.02
Fat (g/100g)	5.51±0.08
Carbohydrates (mg/100g)	6.4±0.02
Calcium (mg/100g)	550±0.08
Protein (g/100g)	16.36±0.45
Energy (kcal/100g)	344±0.20
Iron (mg/g)	9.3±2.11
Vitamin A (mcg/100g)	234±4.87

Antioxidants

Antioxidants are typically used to stop the oxidation process and help preserve food for longer periods of time. Bacteria that act as oxygen scavengers can grow out of control in the presence of antioxidants. Unsaturated fats oxidize, resulting in rancidity, which causes food to smell bad and turn brown (Pandey and Upadhyay 2012).

DPPH Assay

Table 8 DPPH Assay

	Control	Sample	Scavenging %
OD Value	0.928	0.407	56.14%

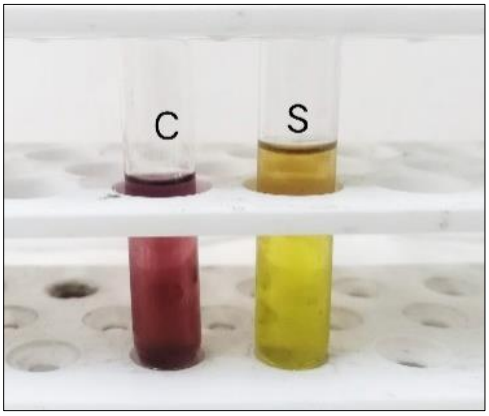

Fig 4 DPPH Assay

According to Rohmah *et al*. (2020), the extracts' ability to reduce DPPH was tested. The extracts' reduction of DPPH causes a decrease in the color of the initial violet. For each extract, two parameters were assigned: the percentage of reduction (Pr) of the DPPH and the concentration in extracts involving 50% reduction. (EC50), which is similar to the study done here which showed 56.14%.

Total phenol and total flavanoid

Since their hydroxyl groups assist them in scavenging free radicals, the total phenolic concentration could be used as a basis for quick screening of antioxidant activity. Redox properties of phenolic compounds allow them to function as antioxidants (Shoib A. Baba 2015).

Table 9 Total phenol and total flavonoid

Total Phenol	53.42 µg/mg
Total Flavonoid	89.15 µg/mg

Phytochemical

Table 10 Phytochemical values

Phytochemical tests	Results
Alkaloids	+
Phenol	+
Flavanoids	+
Coumarins	+++
Terpenoids	-
Quinones	+++
Aminoacid	-
Anthracyanine	-
Saponin	+
Cardiac Glycosides	++

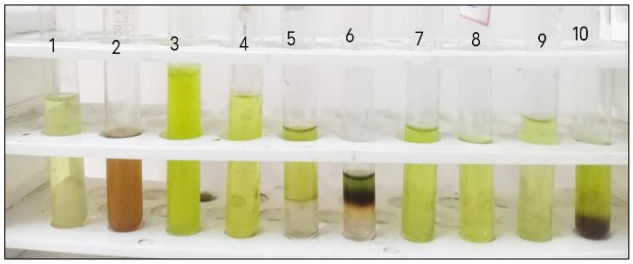

Fig 5 Phytochemicals

Quantitative analysis revealed that the soup cube (++) contained more phytochemicals than the control (*), including alkaloids, phenol, flavonoids, coumarins, terpenoids, and cardiac glycosides. Numerous authors have reported that plant components like phenol, quinones, flavones, tannins, terpenoids, essential oils, and alkaloids have antimicrobial properties. (P Saranraj *et al*. 2016).

Phytochemicals such as alkaloids, phenols, flavonoids, Coumarins, Terpeniods, Quinones, Anthrocyanine, Saponin, Cardiac glycosides, tannins, and beta-carotene in T3 treatment were analyzed both qualitatively and quantitatively. (+++- Indicates Presence in High, ++- Presence in Moderate, +- Presence in Low, - Indicates Absent).

Microbial analysis

The microbial analysis of the prepared sample of *Sesbania grandiflora* incorporated soup cubes were found to be 2.09 x 10^4 CFU/ml of total bacterial count and the total yeast mold count was found to be NIL. This value shows that the product is microbially safe as evaluated by the instant soup mix of Shilpa Yatnatti *et al*. 2018, (Lolge *et al*. 2022).

Table 11 Microbial analysis

Microbes	Treatment (T_1)
TBC	2.09 x 10^4 CFU/ml
TYMC	Nil

Shelf-life analysis

The shelf-life analysis of the freeze-dried soup cube were done for 45 days with the interval of 15 days in between. The TBC and TYMC count at day 15 were found to be 0.727*10^4 and NIL respectively. The counts slightly increased at day 30 with values 1.18*10^4 for both TBC and TYMC which also comes under the permitted value. At day 45, the TBC were found to be 2.27*10^4 and TYMC were 2.54*10^4. All these results have shown that freeze drying the soup cubes had shelf-life of 45 days, without changing the sensory attributes which is very close to the values reported in various research literatures as mentioned in Fahima R *et al*. 2007. (Kozhushko *et al*. 2021).

Table 12 Shelf-life analysis

Analysis	Day 15 (CFU/ml)	Day 30 (CFU/ml)	Day 45 (CFU/ml)
TBC	$0.727*10^4$	$1.18*10^4$	$2.27*10^4$
TYMC	NIL	$1.18*10^4$	$2.54*10^4$

Ethical consideration

This study entitled "Formulation, standardization and quality evaluation of *sesbania grandiflora* leaves incorporated soup cube" has been approved by the Independent Human Ethics Committee (IHEC) with protocol no – SDNBVC/HSC/IHEC/2022/12 conducted by the on October 12, 2022, Department of Home Science, SDNB Vaishnav College for Women, Chromepet, Chennai – 44.

CONCLUSION

To conclude my study, the *Sesbania grandiflora* leaves soup cube is a better option for supplementing calcium through food for astronauts. The results shown from the above research have shown that the freeze-dried soup cubes have retained most of the nutrient content and are also microbially safe. The moisture content of the soup cube has been found to reduce from 100% to 0.36% in the given sample which adds to the extension of shelf-life. The freeze-drying process has a lower impact on food nutrients than other high-temperature dehydration methods. In future, the study can be followed and tested in a microgravity environment to check the quality and sensory characteristics of the soup cube.

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