

## Development and Quality Evaluation of an Herbal Green Sauce from *Centella asiatica* and *Basella alba* L.

J.M.I.N.K. Bandara<sup>1</sup>, P.C. Arampath<sup>2\*</sup> and H.M.A.H. Thilakarathne<sup>3</sup>

### ABSTRACT

*Centella asiatica* (pennywort, “gotu kola”) and *Basella alba* L. (Ceylon green spinach) are locally available leafy greens rich in phytonutrients and phytochemicals. *C. asiatica* has been associated with memory enhancement, anti-carcinogenic effects, and therapeutic use in liver and kidney disorders, while *B. alba* is a valuable source of iron, vitamins, minerals, and antioxidants. Modern lifestyles limit green leaf consumption due to laborious preparation, prompting the development of a convenient herbal green sauce. A consumer survey involving 314 respondents informed formulation, followed by a sensory evaluation of sauces prepared with varying concentrations of leaf extracts (50-70% w/w). Based on sensory evaluation, the formulation containing 55% leaf extract

with a 55:45 ratio of *C. asiatica* to *B. alba* showed the highest overall acceptability and was selected as the optimum formulation combined with water, corn flour, xanthan gum, sugar, salt, vinegar, spices, potassium sorbate, and a permitted artificial colourant to compensate for chlorophyll loss during processing. The herbal sauce contained  $46.29 \pm 1.07\%$  moisture,  $34.22 \pm 0.12\%$  ash,  $1.30 \pm 0.25\%$  crude fat,  $10.35 \pm 0.76\%$  crude protein,  $33.57 \pm 0.15\%$  crude fibre, and  $20.55 \pm 0.27\%$  carbohydrates. The physicochemical properties included pH 4.6, viscosity  $1381.0 \pm 1.2$  mPa.s at 25°C, total soluble solids  $12.08 \pm 1.08$ , and strong green colour. Microbiological analysis confirmed absence of aerobic plate count, yeasts, moulds, coliforms, *Escherichia coli*, and *Salmonella* spp., ensuring product safety. Shelf life was six weeks at  $25 \pm 2^\circ\text{C}$  based on microbial stability and physicochemical parameters. Functional properties of the herbal green sauce were evaluated, revealing total polyphenols of 0.52 mg GAE/g, flavonoids of 0.29 mg QE/g, and antioxidant activity higher than commercial tomato sauces. The sauce demonstrated enhanced nutritional and functional value over conventional condiments, and further studies are recommended to evaluate its therapeutic potential.

<sup>1</sup>Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka

<sup>2</sup>Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka

<sup>3</sup>Bamber and Bruce (Pvt)Ltd, No.95, Piyadasa Sirisena Mawatha, Maradana 01000, Sri Lanka

\*[arampath@agri.pdn.ac.lk](mailto:arampath@agri.pdn.ac.lk)



<https://orcid.org/0000-0003-4471-9343>



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## INTRODUCTION

The modern lifestyle demands pre-prepared or processed foods with time-saving characteristics. The fast foods possess a higher demand among the food categories due to convenience, inexpensive, and desired sensory attributes (Drewnowski and Monsivais, 2020). The table sauce or table condiment is served with the cuisine or as a side dish and become ubiquitous and integral part of the fast-food experience (Ravindran and Kallapurackal, 2012). Tomato ketchup, soy sauce, mustard sauce, hot sauces are the common table sauces which enhance the unique flavour and aroma enhancing the palatability of the certain dishes or the culinary preparation. Sauces are generally considered as less nutrient-dense foods with higher level of calories, fat, and salt (Jaworowska *et al.*, 2013). Hence, the development of convenient healthy foods rich in nutritive value is of utmost importance at present (Nielsen, 2016).

Both *C. asiatica* and *B. alba* L. are well-known herbs. Belonging to the family Apiaceae, *C. asiatica* is a native evergreen perennial widely used for medicinal purposes. The morphotypes of *C. asiatica* include G7 (bush type), G8

(giant type), and others (Chandrika and Kumara, 2015).

*Centella asiatica* possesses significant medicinal properties as an herb in the orient, vegetable, and other culinary applications such as porridges in several parts of South East Asia. The plant is mostly harvested from the wild or commercially cultivated for medicinal and edible purposes (Das, 2011). Triterpenoids such as asiatic acid, has an anti-carcinogenic action, brahmic acid, isobaric acid, brahminoside, and brahmoside are found in the leaves of *C. asiatica* (Han *et al.*, 2020). These compounds are effective in treating dementia, mental disorders, and sleep-deprived anxiety-like behaviour (Tiwari *et al.*, 2008), improve memory, and useful in treatment of Alzheimer's disease (Chen *et al.*, 2016). Due to its memory-improving ability, it is popular among Asians as a cognitive tonic. *C. asiatica* was shown to promote blood circulation to remove blood stasis. Consequently, it is a useful medicinal plant for the prevention of lifestyle-related diseases such as hypertension, cardiopathy and cerebral apoplexy caused by arteriosclerosis (Satake *et al.*, 2007).

The term “spinach” is a versatile term used for various green leafy vegetables in multiple regions of the world (Gayathree *et al.*, 2020). Despite the accurate classification of true spinach as *Spinacia oleracea* within the family Amaranthaceae, in Sri Lanka, the term “spinach” is employed to refer to Malabar spinach, which has two species: *B. alba* L. (Ceylon green spinach) and *Basella rubra* L. (red spinach), which belongs to the family Basellaceae (Chaurasiya *et al.*, 2021). Locally, green spinach is referred to as “Niwithi” (Ganegoda, 2020). *B. alba* L. bears green stems. The immature and recently matured leaves, as well as the stem, collectively referred to as “shoot tops”, are the parts of *B. alba* L. that are edible (Gayathree *et al.*, 2020). Phytonutrients, including a variety of vitamins (vitamins A, C, E, K, B9, niacin, and thiamine), minerals and antioxidants are abundant in the foliage of *Basella* spp. (Kumar *et al.*, 2015) (Gayathree *et al.*, 2020). The non-starchy polysaccharide or type of mucilage found in *B. alba* L. is a significant source of health benefits (Hung and Lai, 2019). Additionally, *B. alba* L. leaves are widely recognized around the world due to their substantial iron content, which is beneficial for anaemia (Fahad *et al.*, 2015). *B. alba* L. leaves are used in

conventional medicine in numerous ways, including the treatment of haemorrhages, impotence, ulcers, skin disorders, and as a laxative for children and pregnant mothers (Adhikari *et al.*, 2012) cold-related infections, cough, and anaemia (Rahmatullah *et al.*, 2010).

The objective of this study was to develop a novel green table sauce utilizing the health-promoting properties of *C. asiatica* (pennywort, “gotu kola”) and *B. alba* L. (Ceylon green spinach). The sauce aimed to enhance palatability and health concerns blended with convenience, nutrient-dense food options that are suitable for modern lifestyles.

## MATERIALS AND METHODS

Consumers’ food habits and selection of food commodities the expectation of novel trend of sauce type were collected via online survey initially. Secondly, a novel leafy sauce was formulated and developed, based on the consumer’s responses. Lastly, a sensory evaluation was conducted to determine the best sauce formulation and shelf-life study by varying leaf extract contents. The leaf extract, 50, 55, 60, 65, and 70% w/w were used as the treatments to develop the sauce. Finally, the best selected

sauce was subjected to sensory evaluation, analysis of the physico-chemical characteristics and microbial safety.

### ***Consumer Survey***

A structured questionnaire survey was conducted via Google forms, yielding 314 responses across diverse age groups, genders, and income categories. The survey captured consumer perceptions, preferences, and purchasing behaviour related to table sauces, as well as expectations toward a novel, green-coloured product with proposed functional health benefits. Domains included demographic information, consumption behaviour, brand preferences, sensory and motivational factors, and product evaluation, with respondents also providing open comments.

### ***Preparation of the Herbal Sauce***

All ingredients, except for the green leaves, were purchased from a reputed supermarket, Kalutara, Sri Lanka. Fresh, mature, and healthy leaves of *C. asiatica* (runner type) and *B. alba* L. were collected from a commercial-scale vegetable grower in Kundasale, Sri Lanka. Damaged and dead leaf

particles were removed, and the leaves were washed thoroughly with potable water to eliminate physical impurities, followed by surface sterilization using chlorine solution (50 ppm) prepared from food-grade chlorine tablets. Residual chlorine was removed by rinsing with potable water, after which the leaves were subjected to chemical blanching in sodium bicarbonate solution (0.1%) at 98°C for 10 s and immediately cooled to ambient temperature in a cold-water bath (Kaushal *et al.*, 2013). Green chilies (*Capsicum annuum* L.) underwent the same sterilization and blanching process. Spices including black pepper (*Piper nigrum*), cumin (*Cuminum cyminum*), cinnamon (*Cinnamomum verum*), cloves (*Syzygium aromaticum*), ginger (*Zingiber officinale*), garlic (*Allium sativum*), and nutmeg (*Myristica fragrans*) were purchased from the same supermarket and cleaned to remove physical contaminants. Leaf extracts of *C. asiatica* (CAE) and *B. alba* L. (BAE) were prepared separately using a blender (Thunder Bullet, BL216ABC) and combined in a 1:1 ratio (w/w). The resulting pure extract (without pulp) was then incorporated with the other constant ingredients to prepare green sauce samples with five inclusion levels: T1 – 50%, T2 – 55%, T3 – 60%, T4

– 65%, and T5 – 70% (w/w). Water was added only during the sauce preparation stage, not during extract collection. A control sample was not included due to the absence of comparable commercial products. Sensory evaluation was conducted with 32 semi-trained panellists under controlled laboratory conditions using a 7-point Hedonic scale to assess aroma, mouthfeel, flavour, colour, and overall acceptability.

#### ***Optimization and Thermal Processing of the Herbal Sauce***

The most acceptable formulation was further refined by varying the CAE:BAE ratio (F1 - 45:55, F2 - 50:50, F3 - 55:45, w/w). Percentages of spices, corn flour, xanthan gum, sugar, salt, vinegar, and artificial green colour (INS 142) were adjusted to optimize physicochemical and sensory properties. The herbal sauce was prepared by mixing the selected leaf extract blend with spices, salt, and sugar, and heating at 55-60°C for 2-3 min with continuous stirring. Xanthan gum and sugar were added before reaching 65°C, followed by dissolved potassium sorbate and corn flour. The mixture was heated at 65–70°C until reaching 32° Brix, measured using a

digital refractometer (Model PAL-1, Atago Co. Ltd., Tokyo, Japan). Heating was stopped once the target 32° Brix was achieved, and the sauce was hot-filled into sterile glass bottles (350 mL), hermetically sealed, and stored until further analysis.

#### ***Determination of Initial Microbial Quality of the Herbal Sauce***

Total plate count (ISO 4833-1:2013), yeast and mould counts (ISO 21527-2:2008), total coliform count (ISO 4832:2006), *E. coli* count (ISO 7251:2005), *Salmonella* spp. (ISO 6579-1:2017) in the developed herbal sauce were determined using test procedures of the ISO standards.

#### ***Determination of Proximate Composition of the Leaf Extract and the Herbal Sauce***

Moisture content (Ranganna, 1986), ash content (AOAC: 925.11.), crude fat (AOAC: 922.06), crude protein (AOAC: 920.25), crude fibre content (AOAC: 920.86) and carbohydrate (Ranganna, 1986) were determined from the samples of leaf extract and the novel herbal sauce.

### ***Determination of Physico-chemical Properties***

The colour of the herbal sauce was determined using the CIELAB (CIE L\*a\*b\*) colour space method (Brainard, 2003). A 50 mL well stirred sample was poured into a glass Petri dish (diameter: 9.25 cm, height: 1.5 cm) placed on a white base, and measurements were taken using a digital chromameter (KONICA MINOLTA CR-410, Japan). Results were expressed as L\* (lightness, ranging from 0 = black to 100 = white), a\* (hue between red and green), and b\* (hue between yellow and blue) values.

Viscosity was measured using a Brookfield viscometer (Model RV-II+, Massachusetts, USA) with spindle number SP 03 at a rotational speed of 60 rpm. A 500 mL volume of the sauce sample was tested in triplicate, and the ambient temperature during measurement was maintained at  $26 \pm 0.2^\circ\text{C}$ .

The pH of the sauce was determined using a pH meter (Apera, pH700). A 50 mL sample was analysed with a calibrated electrode, standardized using buffer solutions of pH 4, 7, and 10 at  $25^\circ\text{C}$ .

### ***Determination of Functional Properties***

Antioxidant content of the sauce was determined by total polyphenolic content (TPC) using Folin- Ciocalteu reagent (Singleton *et al.*, 1999), and total flavonoid content (TFC) by spectrophotometric method (Siddhuraju and Becker, 2003). DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS[2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)] diammonium salt assays, by (Blois *et al.*, 1958) and (Re *et al.*, 1999) respectively used to determine the antioxidant activity.

### ***Determination of the Shelf Life of Herbal Sauce***

Titrateable acidity (AOAC: 942.15), pH (AOAC: 981.12), water activity (ISO 18787:2017), total soluble solids (AOAC: 932.12) (refractometry method), total plate count, and yeast and mould counts were determined at two-week intervals over a nine-week period from samples stored under ambient laboratory conditions ( $25 \pm 2^\circ\text{C}$ ), monitored using a digital thermometer (Perera and Pavitha, 2017).

### Statistical Analysis

Statistical analysis of sensory scores was performed using the Friedman test ( $p < 0.05$ ) and Wilcoxon signed rank test ( $p < 0.05$ ) in SPSS (Version 25.0, IBM Corp., Armonk, NY, USA).

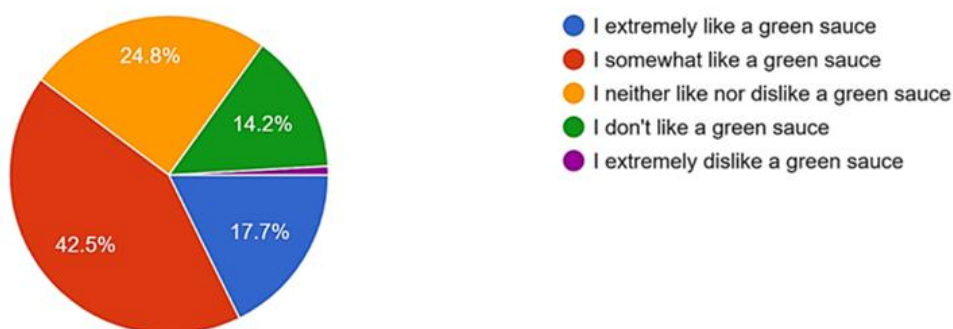
## RESULTS AND DISCUSSION

### Consumer Survey

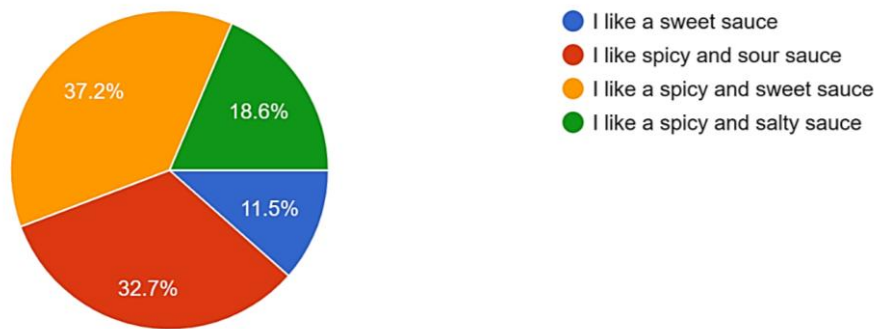
The consumer survey indicated that 60.2% of respondents expressed a favourable preference toward the proposed green-coloured herbal sauce (Figure 1), suggesting openness to unconventional product appearances when accompanied by perceived nutritional and functional health benefits. In terms of sensory expectations, 37.2% of respondents anticipated sweet and spicy attributes (Figure 2), reflecting a preference for balanced flavour profiles that combine

novelty with familiar taste experiences. These insights were incorporated into the formulation process to ensure that product development aligned with consumer expectations.

The findings emphasize that sensory qualities; particularly taste and aroma; play the most decisive role in shaping consumer acceptance of table sauces, while functional health benefits are recognized as valuable however, act as secondary factors influencing purchase decisions. The favourable response to the green colouration highlights potential market acceptance, although mixed opinions suggest that visual presentation may remain a barrier requiring targeted marketing strategies. Cultural influences were also evident, as Sri Lankan consumers prioritized affordability and taste, with functional health claims serving as an added advantage rather than a primary driver.



**Figure 1:** Respondents preference for a green sauce



**Figure 2:** Respondents expected preference for the flavour of the novel green sauce

Overall, the results demonstrate that successful product positioning must balance sensory quality, affordability, and functional health benefits to ensure consumer acceptance.

#### ***Preparation of the Herbal Sauce and Sensory Evaluation***

Chemical blanching in sodium bicarbonate solution (0.1%) for 10 s at 98°C was carried out to arrest the enzyme activity of peroxidases (Kaushal *et al.*, 2013). This pre-treatment is effective in inactivating peroxidase enzymes and reducing antinutritional factors. However, thermal treatments can also result in the loss of phytonutrients in canned fruits due to diffusion and leaching (Arampath and Dekker, 2021). The extent of nutrient loss is influenced by the duration of heating and the particle

size of the product, with ascorbic acid being particularly sensitive to thermal degradation. The use of chemical agents during blanching has been shown to mitigate these concerns (Gupta *et al.*, 2008). Recent studies confirm that hot water blanching at 98°C for 10 s is sufficient to inactivate peroxidase activity, whereas steam blanching may not achieve the same level of inactivation. Moreover, chemical blanching has a protective effect on chlorophyll retention, with sodium bicarbonate treatment (0.1%) reported to preserve maximum chlorophyll and antioxidant concentrations in *Colocasia* leaves (Kaushal *et al.*, 2013).

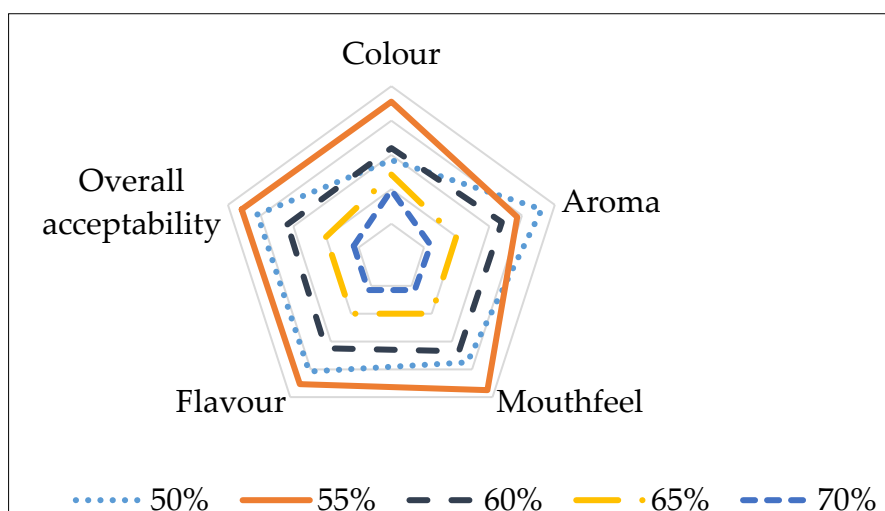
Corn flour, xanthan gum, sugar, salt, vinegar, and artificial green colour (INS 142) were not incorporated during the extract collection stage because their functional roles are specific to sauce

formulation rather than extraction. Corn flour and xanthan gum act as thickening and stabilizing agents, sugar and salt contribute to flavour balance, vinegar provides acidity and preservation, and INS 142 ensures consistent product colour. Adding these ingredients only during the sauce preparation stage allowed the pure leaf extract to be collected without interference, while ensuring the final product achieved the desired physicochemical stability, sensory quality, and consumer acceptability.

Among the prepared formulations, the green sauce sample produced using treatment T2 (55% w/w total leaf extract, prepared with a 1:1 ratio of *C. asiatica* extract to *B. alba*

extract) was identified as the most acceptable. This formulation recorded the highest mean scores for colour, aroma, mouthfeel, flavour, and overall acceptability compared to other treatments (Figure 3). Statistical analysis confirmed that these differences were significant at  $p < 0.05$ , as determined by the Friedman test followed by the Wilcoxon signed-rank test.

Since the total leaf extract was prepared at a 1:1 ratio of *C. asiatica* and *B. alba* extracts, a lower mean score was recorded for mouthfeel which was attributed to the lower viscosity of *C. asiatica* extract affecting the overall texture.

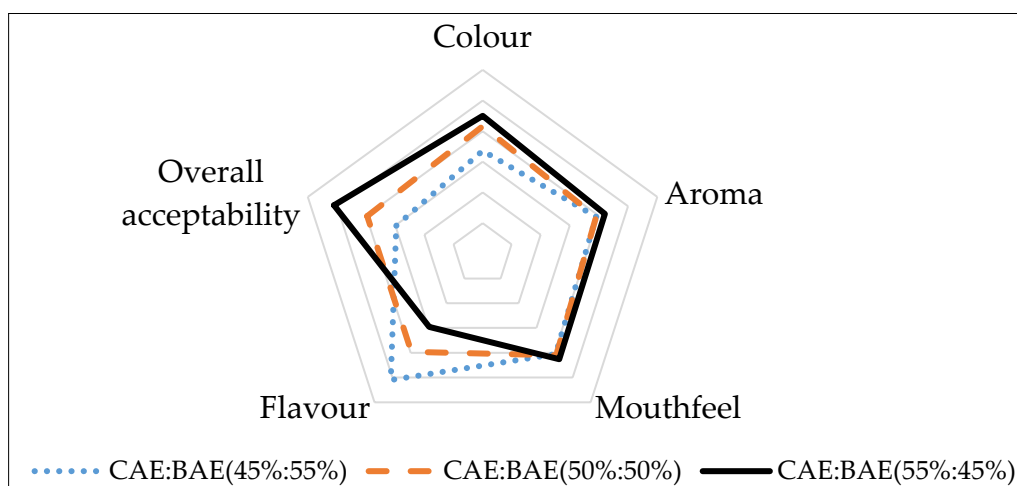


**Figure 3:** Mean scores of sensory attributes of green leave of the herbal sauce (1: dislike extremely, 7: like extremely)

Consequently, the formulation containing 50% (w/w) leaf extract was less preferred compared to the 55% formulation, as the reduced viscosity negatively affected the dipping quality of the sauce. Sauces with higher concentrations of leaf extract were also found to have less desirable flavour among panellists (Figure 4). This observation is consistent with Apichartsrangkoon *et al.* (2009), who reported that  $\gamma$ -Terpinene, a compound present in heat-treated *C. asiatica* extract, contributes to bitterness, thereby reducing consumer acceptability at higher concentrations. Volatile oils, which contribute to the aroma of the sauce, are prone to degradation and evaporation during thermal processing (Su and Wiley, 1998). The aroma of *C. asiatica* extract is primarily attributed to volatile compounds such as  $\beta$ -caryophyllene, humulene, and  $\alpha$ -copaene (Apichartsrangkoon *et al.*, 2009), some of which may interact with macromolecules through hydrophobic effects, reducing aroma release (Jouquand *et al.*, 2004). Interestingly, formulations with higher spice concentrations but lower leaf extract content achieved higher mean ranks, suggesting that spices enhanced sensory appeal.

Both *C. asiatica* and *B. alba* leaves are chlorophyll-rich; however, heat treatment caused chlorophyll degradation, with *C. asiatica* extract appearing more heat-sensitive and prone to discolouration. Chemical (alkaline) blanching was applied to mitigate this effect. Loss of green colour in food products is associated with pheophytin formation, where  $Mg^{2+}$  in chlorophyll is replaced by  $H^+$  (Scipioni *et al.*, 2010). The use of sodium bicarbonate (0.1%) solution provided an alkaline environment (pH 7-9), which stabilized chlorophyll and minimized discolouration (Koca *et al.*, 2007).

Although the formulation contained synthetic food-grade colourant (INS 142), its inclusion was limited to colour standardization due to chlorophyll degradation during processing. The functional and nutritional characteristics of the sauce were primarily derived from *C. asiatica* and *B. alba* extracts. Future product development should investigate natural alternatives for colour stabilization to better align with the concept of a natural herbal functional food.



**Figure 4:** Mean scores of the sensory attributes of herbal sauce (1: dislike extremely, 7: like extremely)

Based on sensory evaluation of F1, F2, and F3 treatments, the 55:45 (CAE:BAE) ratio was identified as the most acceptable formulation. This was confirmed statistically using the Friedman test ( $p < 0.05$ ) and Wilcoxon signed-rank test ( $p < 0.05$ ) (Figure 4). Although the 55:45 CAE:BAE ratio achieved the highest mean rank for aroma, mouthfeel, colour, and overall acceptability, its flavour score was comparatively lower. This outcome may be explained by the higher proportion of *C. asiatica* extract, which contains volatile compounds such as  $\gamma$ -Terpinene that impart bitterness upon thermal processing.

These phytochemical characteristics, while contributing to

functional value, may adversely affect flavour perception, underscoring the need to balance extract ratios to optimize both nutritional functionality and sensory appeal. Ultimately, the optimized formulation consisted of 55% (w/w) herbal blend (55:45 CAE:BAE), combined with other ingredients: water (18.6%), corn flour (5.4%), xanthan gum (0.4%), sugar (6.7%), salt (0.4%), vinegar (1%), synthetic food-grade colourant (E142, 4 mL/kg), pepper (0.4%), cumin (1.2%), cinnamon (0.4%), green chili (4.6%), ginger (3.0%), garlic (0.8%), nutmeg (0.8%), and potassium sorbate (1000 ppm) (Figure 5). Corn starch and xanthan gum were incorporated as thickening and stabilizing agents to mitigate syneresis by binding water molecules with polysaccharide

hydrocolloids, thereby increasing viscosity and improving product stability (Sikora *et al.*, 2008).



**Figure 5:** An image of prepared herbal sauce

#### ***Determination of Initial Microbial Quality of the Herbal Sauce***

The microbiological analysis of the herbal sauce highlights its exceptional hygiene and safety standards. The aerobic plate count, yeast and mould count, total coliform count, and *E. coli* count were tested. Initially there were no detectable microorganisms in the sauce (Table 1). The absence of aerobic microorganisms (0.0 log CFU/g) reflects positive hygienic processing practices and food safety, encompassing excellent good manufacturing practices (GMP),

meticulous handling, effective sanitation, and a well-controlled processing environment. The results were especially significant for the experimented herbal sauce as a perishable product, which requires a high degree of microbiological quality and food safety.

The absence of yeasts and Molds (0.0 log CFU/g), typically regarded as zero or no spoilage indicators, further underscores the quality of the product. This suggests that the herbal sauce was protected from environmental factors conducive to fungal growth, such as high humidity or poor storage conditions. Additionally, the absence of coliforms (0.0 MPN/g), which serves as an indicator of faecal contamination and overall hygiene, confirms that the raw materials and water used in production were clean and that strict sanitary measures were followed throughout the manufacturing process.

*E. coli*, a specific type of coliform critical for assessing food safety, was also undetectable, signifying that the product satisfied the essential microbiological safety standards and free from direct faecal contamination.

**Table 1:** Initial microbial count of the developed herbal sauce

| Parameter                         | Initial microbial count |
|-----------------------------------|-------------------------|
| Aerobic plate count (log CFU/g)   | 0.0                     |
| Yeast and mould count (log CFU/g) | 0.0                     |
| Total coliform count (MPN/g)      | 0.0                     |
| <i>E. coli</i> count (MPN/g)      | 0.0                     |
| <i>Salmonella</i> spp./ 25g       | Absent                  |

Moreover, the sauce tested negative for *Salmonella* spp. in 25g of the sample, a critical finding that aligns with internationally recognized standards, such as the Codex Alimentarius. This result highlights the rigorous hygiene and safety protocols implemented during production and storage, ensuring the product is safe for consumption. Overall, these findings confirm that the herbal sauce is of excellent quality, with stringent measures effectively mitigating risks associated with microbial contamination.

#### ***Determination of Proximate Composition of the Leaf Extract and the Herbal Sauce***

The proximate composition of the herbal sauce is presented in Table 2. The moisture content of the sauce ( $46.29 \pm 1.07\%$ ) was lower than the leaf extract ( $53.57 \pm 1.23\%$ ), attributed to the

addition of soluble solids and moisture loss during thermal processing. The total soluble solids (TSS) content of the sauce was  $12.08 \pm 1.08\%$ , significantly higher than the leaf extract ( $2.57 \pm 1.21\%$ ) and low crude fat content ( $1.30 \pm 0.25\%$ ), considered favourable for health. The higher ash content ( $34.22 \pm 0.12\%$ ) observed due to the mineral contribution of the added spices. The crude fibre content of the sauce was  $33.57 \pm 0.15\%$ , representing water insoluble dietary fibre composed of cellulose, lignin, pentosans, and other cell wall materials. Such fibre intake supports digestive health and promotes the growth of beneficial gut microbiota.

The crude protein content ( $10.35 \pm 0.76$  g/100 g) was notably higher than that reported for commercial tomato and chilli sauces (1.2 - 3.0 g/100 g), highlighting the nutritional advantage of the herbal formulation.

**Table 2:** The proximate composition of the herbal blend (CAE: BAE, 55:45) and the herbal sauce prepared with 55% w/w herbal extract

| Constituent          | Leaf extract blend<br>( <i>C. asiatica</i> and <i>B. alba</i> L.) | Herbal sauce |
|----------------------|-------------------------------------------------------------------|--------------|
| Moisture content (%) | 53.57 ± 1.23                                                      | 46.29 ± 1.07 |
| Ash (%)              | 38.18 ± 0.17                                                      | 34.22 ± 0.12 |
| Crude fat (%)        | 1.74 ± 0.46                                                       | 1.30 ± 0.25  |
| Crude protein (%)    | 18.93 ± 0.84                                                      | 10.35 ± 0.76 |
| Crude fibre (%)      | 38.08 ± 0.19                                                      | 33.57 ± 0.15 |
| Carbohydrate (%)     | 3.06 ± 1.02                                                       | 20.55 ± 0.27 |

#### ***Determination of Physico-chemical Properties***

The total soluble solids (TSS) content of the sauce was  $12.08 \pm 1.08\%$ , significantly higher than the leaf extract ( $2.57 \pm 1.21\%$ ). Total soluble solids of commercial tomato and chili sauces is typically contained 18-27% w/w, whereas the developed herbal sauce possessed 12.08%, reflecting a comparatively lower sugar content. Total soluble solids include sugars, water soluble proteins, amino acids, food colourants, salts, and other organic compounds, and the total soluble content exceeded the sugar fraction alone. The TSS value of  $12.08 \pm 1.08$  g/100 g was considerably lower than the sugar content typically found in local market sauces ( $\geq 30$  g/100 g).

The pH of the herbal sauce was measured as 4.6 at  $25 \pm 2^\circ\text{C}$ , which falls within the recommended range for ensuring microbial safety, as growth of *Clostridium botulinum* is inhibited below pH 4.6 (Rahman *et al.*, 2015). Comparable pH values have been reported in *Gymnema sylvestre* based sauces stabilized with sodium benzoate, which maintained values around 4.5 and complied with international standards for acidic condiments (Perera and Pavitha, 2017).

Colour parameters determined using the CIELAB method were  $L^* = 36.72 \pm 0.69$ ,  $a^* = -100.44 \pm 0.22$ , and  $b^* = -100.88 \pm 0.47$ . The negative  $a^*$  value indicates a strong green hue, attributable to the chlorophyll content of the herbal leaves. Similar findings have been reported in pesto

formulations incorporating moringa leaves, which enhanced green chromaticity and reduced lightness values (Brito *et al.*, 2025). Mint-coriander sauces also demonstrated high chlorophyll retention, contributing to desirable green colouration and consumer acceptability (Kashyap *et al.*, 2016).

The viscosity of the herbal sauce was  $1381.0 \pm 1.2$  mPa·s at 26°C. This value is comparable to the viscosity range reported for commercial chili sauces in Malaysia (1207.01–2386.72 mPa·s at 25°C) (Mohammad Azmin and Mat Nor, 2020). Viscosity is a critical quality parameter influencing mouthfeel and consumer perception. The higher viscosity observed in the present formulation compared to *Gymnema sylvestre* sauces thickened with sweet potato flour (700–800 mPa·s) (Perera and Pavitha, 2017) and mint-coriander sauces stabilized with guar gum and CMC (745–750 mPa·s) (Kashyap *et al.*, 2016) may be attributed to the specific composition of the herbal extract and the interaction of polysaccharides with added stabilizers.

Overall, the physico-chemical properties of the herbal sauce's pH stability, green-coloured intensity, and

viscosity are consistent with previous studies on functional sauces formulated with unconventional leafy greens. The pH ensures microbial safety, the colour parameters highlight chlorophyll retention, and the viscosity supports a desirable texture comparable to that of commercial sauces, reinforcing the potential of herbal sauces as functional condiments.

### **Determination of Functional Properties**

The functional properties of the herbal green sauce were assessed through quantification of polyphenolic and flavonoid contents and evaluation of antioxidant activity. As presented in Table 3, the total polyphenolic content (TPC) was  $0.52 \pm 0.02$  mg GAE/g, while the total flavonoid content (TFC) was  $0.29 \pm 0.03$  mg QE/g. These values are lower than those reported for raw *C. asiatica* (2.82 mg/g GAE) and *B. alba* L. (3.57 mg/g GAE) leaf extracts, indicating that extraction and thermal processing steps such as blanching and heating reduced the retention of bioactive compounds. Nevertheless, the herbal sauce demonstrated higher phenolic content than commercial tomato sauces (0.32 mg/g GAE) (Boateng *et al.*, 2008), suggesting that

the product offers a relative nutritional advantage compared to conventional condiments.

The discrepancy between total phenolic content (0.52 mg GAE/g) and antioxidant activity measured by DPPH (60.36 mg TE/g) and ABTS (10.49 mg TE/g) assays reflects differences in assay principles and the phytochemical composition of the sauce. While the Folin–Ciocalteu method quantifies only phenolic compounds, DPPH and ABTS assays capture radical scavenging activity from both phenolic and non-phenolic antioxidants. The relatively high DPPH value suggests that lipophilic compounds such as triterpenoids and volatile oils from *C. asiatica* contributed significantly to

radical scavenging capacity, whereas ABTS values were lower due to its greater sensitivity to hydrophilic antioxidants. Thus, the large ratio between DPPH and ABTS results is attributable to the dominance of lipophilic antioxidants and the differential responsiveness of the assays.

Taken together, the data in Table 3 demonstrate that the herbal sauce preserves functional properties derived from its leafy components and added spices. While the values are lower than those of raw extracts, they remain superior to conventional sauces, supporting the sauce's potential as a functional food product with enhanced nutritional and bioactive qualities.

**Table 3:** Results for the antioxidants content and antioxidant activity of the herbal sauce

| Parameter             | Value        |
|-----------------------|--------------|
| TPC (mg GAE/g)        | 0.52 ± 0.02  |
| TFC (mg GAE/g)        | 0.29 ± 0.03  |
| DPPH assay (mg TE /g) | 60.36 ± 0.69 |
| ABTS assay (mg TE /g) | 10.49 ± 0.41 |

Data represented as mean ± SEM. TPC and TFC; n=4 each

\*DPPH assay: 2,2-diphenyl-1-picrylhydrazyl

\*ABTS assay: 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid

Data represented as mean ± SEM (n=4) IC<sub>50</sub>Trolox for DPPH: 6.54 ± 0.15 µg/mL

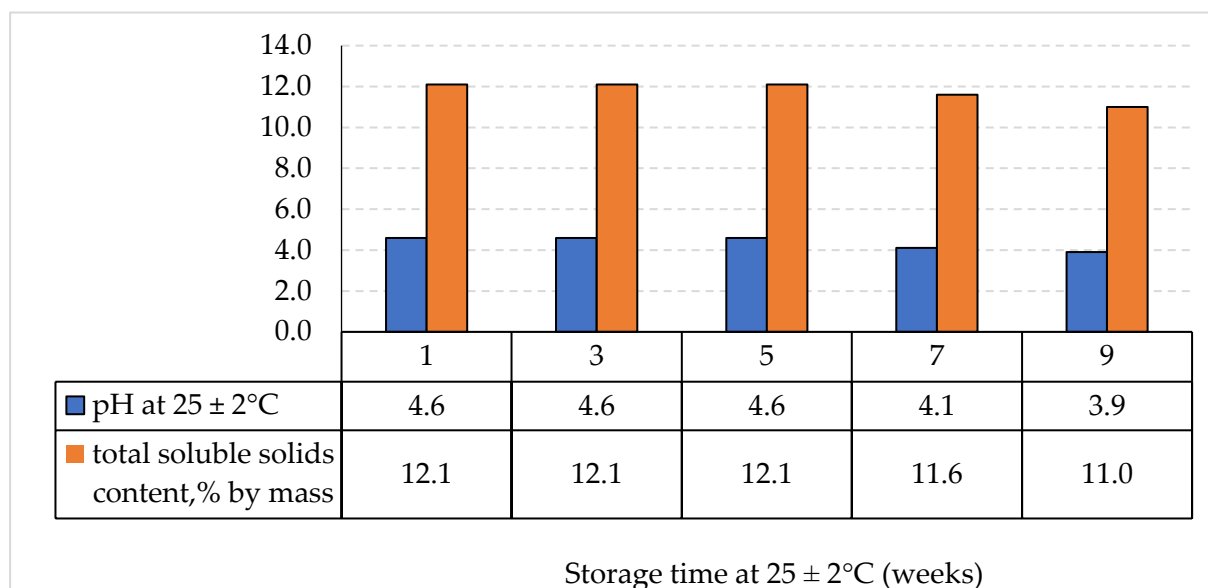
Data represented as mean ± SEM (n=4) IC<sub>50</sub>Trolox for ABTS: 4.96 ± 0.04 µg/mL

### Determination of the Shelf Life of Herbal Sauce

According to previous studies, *Clostridium botulinum* is inhibited at pH 4.6, which corresponded to the initial pH of the herbal sauce. However, spoilage bacteria are optimally suppressed at pH 4.3. Since the initial pH of the sauce was slightly above this threshold, the potential for fungal contamination and yeast fermentation was present (AL-Mamun *et al.*, 2018). As shown in Figure 6, a reduction in pH was observed after the sixth week of storage, likely due to organic acid production during microbial fermentation (Fleet, 2011). This decline

in pH coincided with a gradual reduction in TSS, reflecting the utilization of soluble substrates in biochemical degradation reactions.

Microbiological analysis confirmed that the aerobic plate count (APC) and yeast and mould counts remained undetectable up to the fifth week of storage (Table 4). By the seventh week, microbial growth became evident, with APC reaching 3.1 log CFU/g and yeast and mould counts at 2.4 log CFU/g. At nine weeks, these values increased further to 4.3 log CFU/g and 3.4 log CFU/g, respectively.



**Figure 6:** Change of the total soluble solids content (% by mass) and the pH of the herbal sauce during storage

**Table 4.** Change of the aerobic plate count (APC) and the yeast and mould count (YM) in the herbal sauce during storage

| Storage time at 25°C<br>(weeks) | Aerobic plate count (log<br>CFU/g) | Yeast and mould count<br>(log CFU/g) |
|---------------------------------|------------------------------------|--------------------------------------|
| 1                               | ND                                 | ND                                   |
| 3                               | ND                                 | ND                                   |
| 5                               | ND                                 | ND                                   |
| 7                               | 3.1                                | 2.4                                  |
| 9                               | 4.3                                | 3.4                                  |

The delayed onset of microbial growth can be attributed to the preservative effect of potassium sorbate, which disrupts microbial metabolism by accumulating protons and anions within the cell. Its activity is most effective at pH 3.0 - 4.5 ( Perera and Smith, 2007; Musyoka *et al.*, 2018), explaining why microbial inhibition was maintained during the early storage period but diminished as pH values shifted.

Corn flour, added as a stabilizer, minimized acid hydrolysis and syneresis, while water activity (0.98) and titratable acidity (0.1% w/w acetic acid) remained constant throughout storage. Together, these findings demonstrate that the sauce maintained microbial stability for five weeks, after which spoilage organisms began to

proliferate, consistent with the observed physicochemical changes.

## CONCLUSIONS

This study successfully formulated and processed a novel herbal green sauce using a blend of *C. asiatica* and *B. alba* L. leaf extracts, together with other ingredients including potassium sorbate (1,000 ppm) as a preservative. The product demonstrated acceptable sensory properties, physicochemical stability, and microbiological safety for up to six weeks under ambient storage conditions ( $25 \pm 2^\circ\text{C}$ ). These findings confirm the feasibility of incorporating underutilized, nutrient-rich plants into value-added food products with practical shelf-life stability.

Nutritional analysis revealed that the herbal sauce contained markedly

lower levels of crude fat ( $1.30 \pm 0.25\%$ ), TSS ( $12.08 \pm 1.08\%$ ), and carbohydrates ( $20.55 \pm 0.27\%$ ) compared to commercially available tomato and chili sauces. In contrast, antioxidant content was significantly higher, underscoring its potential as a functional food with added health benefits. Collectively, these attributes position the herbal sauce as a healthier alternative to conventional condiments, aligning with consumer demand for plant-based, sustainable, and bioactive-rich foods.

Beyond product formulation, the study validates the nutritional and functional properties of *C. asiatica* and *B. alba* L., bridging traditional ethnobotanical knowledge with modern food science. The demonstrated six weeks of shelf stability and consumer acceptability highlight opportunities for diversification within the food industry, particularly in the development of functional condiments that support preventive health strategies.

Future research should focus on therapeutic studies to substantiate specific health claims, comprehensive profiling of phytochemicals, and clinical trials to establish efficacy and

safety. Product innovation may also explore reduced synthetic additives, natural colourants, alternative preservatives, and advanced packaging technologies to extend shelf life. Consumer acceptance studies and commercial feasibility assessments will be essential for successful adoption.

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