Biochemical, Organoleptic and Antimicrobial Assessment of Asparagus (Asparagus Officinalis)

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Abstract: Asparagus is a spring vegetable, considered as a delicacy in the vegetable world. It is a nutrient dense, low calorie vegetable with no fat. It has a distinct savory flavor. Asparagus contains unique array of phytonutrients and is an important source of inulin, the digestive support nutrient. It has high amounts of vitamin A, vitamin C, potassium, iron and calcium. It also acts as diuretic. The benefits of asparagus are unexplored in traditional Indian cuisine which makes it interesting to explore its nutritional, biochemical and organoleptic profile. Asparagus has been found to contain anti-fungal, anti-cancer activities. It is a rich source of fiber and helps to maintain a healthy digestive system. Asparagus is believed to balance and rejuvenate the reproductive system in women. Analysis of proximate principles of asparagus was carried out and nutritional profiles of raw and cooked asparagus spears were established. A novel edible product was developed and its organoleptic analysis was carried out with help of semi-trained panel of sensory evaluators. The data obtained was analyzed to check the acceptability of asparagus and its product. Critical control points were established during the process of novel product development and hazard analysis at critical control points was carried out. Anti-microbial activity of asparagus extracts was assessed against bacterial and fungal species which could be linked to its role in maintain a healthy digestive system and gut flora. A simulated product development analysis was carried out. Future prospects include isolation of flavonoids, glycosides and analysis of enzyme profile (invertase activity) of asparagus. The use of asparagus as a functional food and development of novel products keeping in mind the nutritional profile and organoleptic acceptance needs to be explored.

I. Introduction

Food is considered to be the cradle of human sustenance. The evolution of the human species was considered complete when mankind attained the knowledge of procuring and producing food. The cornucopia of edible plants in nature has contributed to the nourishment of mankind across ages and cultures. In today's global society, the consortium of cultures has provided a great exposure to non-indigenous cuisines. The Indian palate is thus fledging towards savoring novel cuisines. This has generated a greater interest in understanding and exploring the novelty of unfamiliar ingredients. This research aims to explore the unique biochemical and organoleptic profile of Asparagus, a non-indigenous ingredient that is slowly creating a niche in the Indian palate.

Asparagus:

Asparagus officinalis has been cultivated and harvested from the wild for thousands of years and has become an economically important crop. It is depicted on Egyptian tombs dating from the 4th century BC and evidence suggests it was cultivated in ancient Rome. The common name Asparagus derives from the Greek *asparagos* (and originally the Persian *asparag*) meaning sprout or shoot, referring to the succulent shoot tips (spears) that emerge in spring. The specific epithet *officinalis* means 'of the dispensary' in Latin, alluding to the medicinal properties of the plant. In all species of the genus *Asparagus*, what appear to be the leaves are in fact modified stems (cladodes or phylloclades); the true leaves are the scale-like structures on the stem.

The primary use of asparagus is as a vegetable, and it is widely cultivated and eaten. As a fresh vegetable, it is lightly boiled, steamed, blanched or grilled and often served with butter. Asparagus is not productive until the third year of growth, and its yield declines after a few seasons. This limited productivity contributes to its status as a high- value crop. Chemical constituents of *Asparagus officinalis* include glycosides, flavonoids and the amino acid asparagine.

Varieties of asparagus:

A significant amount of genetic diversity exists among asparagus species. The most abundant species consumed and cultivated worldwide is *Asparagus officinalis and Asparagus racemosus*. However, to the naïve consumer, the varieties of this vegetable are synonymous with color.

The most commonly consumed species of Asparagus is green in color. However, a variation of this species is the white asparagus, obtained when asparagus growers allow the shoots to grow beneath the soil. This process prevents sunlight from falling on the shoots and results in asparagus shoots that are white in color. However, other varieties of asparagus can also be purple in color. These varieties typically have higher sugar content than green and white varieties and for this reason have a sweeter taste.

II. Review To Literature

A detailed and exhaustive review of scientific literature highlights the following aspects of Asparagus as a research endeavor:

- 1. Asparagus is abundant in proteins, which is abundant in all essential amino acids. These essential amino acids constituted 40–43% of the total amino acid content in Asparagus.
- 2. Prominent phytochemicals in asparagus include rutin, flavones, saponins and polysaccharides. These phytochemicals are rendered as potential antioxidant as they exhibit good removal effects on hydroxyl radicals, superoxide anion free radicals, and DPPH free radicals in-vitro.
- 3. Pigments predominant in Asparagus include xanthophylls (neoxanthin, violaxanthin, lutein and eaxanthin), carotenoids (β -carotene) and chlorophylls a, b, b' in addition to pheophytins *b* and *a*.
- 4. Therapeutic effects of Asparagus are not limited totumor suppressing properties, reduction of blood LDL, anti-fatigue, and enhancement of immunity.
- 5. The hypolipidemic effect of Asparagus extract is comparable to the phytochemicals in oats. Superoxide dismutase activity and the total antioxidant capacity were evidently increased on consumption of Asparagus extract, which further contributes to its hypolipidemic effects.
- 6. Asparagus is effective in the management of hypertension as the various phytochemicals act as potent Angiotensin Converting enzyme (ACE) inhibitors.
- 7. The organoleptic profile of Asparagus (both green and white varieties) is rendered favorable by most panelists.
- 8. The post-harvest respiratory activity (rate of production of carbon dioxide) is a good indicator to predict the shelf life of asparagus.
- 9. Psychotropic bacteria are one of the major agents causing spoilage of Asparagus. Thus, modern processing technologies that reduce the growth of psyochotropic bacteria like vacuum packaging are rendered more effective in elevating the shelf-life of asparagus.
- 10. RAPD Analysis of Asparagus points towards significant genetic diversity in various species of Asparagus.

A) PROXIMATE ANALYSIS:

III. Materials And Methods

This section of the research endeavor focused on identifying, characterizing and quantifying the various nutritional principles of Asparagus.

i) Samples utilized:

Locally procured raw Asparagus was used for analysis. A portion of the raw sample was cooked in boiling water with a pinch of salt for 3 minutes to yield the cooked sample. Both the raw and cooked samples were utilized separately for proximate analysis.

ii) Processing of samples for Analysis:

The samples of raw and cooked asparagus were processed to yield an aqueous extract and an ash solution. The steps involved in the preparation of these solutions is detailed as follows:

Preparation of Ash solution:

This involved the following distinct steps:

Drying of sample: A predetermined amount (usually in the range of 5-10g) of raw asparagus and cooked asparagus was weighed and placed in a clean, dry, empty petri plate. The plate was then placed in a hot air oven set at 100-110°C for around 45 minutes to an hour until the sample was dry. The plate was placed in a desiccator and its weight was recorded. The sample was dried subsequently at 90°C for 10 minutes intervals under the same conditions until the constant weight was obtained.

Incineration of sample: The entire contents of the petri plate were transferred into a clean, dry, pre-weighed empty silica crucible. The crucible was then placed in the incinerator to allow ashing of the sample. To facilitate evaporation of carbon from the sample, few drops of 0.1N HNO3 was added during incineration as an oxidizing agent. The sample was incinerated until a white ash residue was left behind. The crucible was then cooled in a desiccator.

Extraction of ash: The ash residue in the crucible was extracted by dissolving it in 1.0ml of 1:1 HCl and transferred into a 50ml of standard flask. Further, three washings of 0.5 ml of 1:1 HCl each to completely extract all ash. The extracted ash in the 50 ml ash solution was diluted with distilled water up to 50 ml mark in the standard flask. This resultant ash solution was used for the analysis of minerals in the samples.

Preparation of Aqueous extract:

Around 1g of the sample was taken in the mortar and pestle. Some amount of distilled water was added to the sample and it was macerated thoroughly. The aqueous phase was transferred into a 50 ml of standard flask after filtration through a muslin cloth. Subsequent extracts were collected in the same manner and the extract was diluted to the 50ml mark with distilled water. This aqueous extract was used in the estimation of carbohydrates, reducing sugars, proteins, vitamin C, and other nutrients in the sample.

Proximate analysis:

The following proximate principles were analyzed from the two samples of Asparagus-

- 1. Estimation of Total carbohydrates by Anthrone method.
- 2. Estimation of Proteins by Folin Lowry method.
- 3. Estimation of Crude Fibre by Ashing method.
- 4. Determination of Moisture content.
- 5. Determination of Ash content.
- 6. Estimation of Calcium by EDTA method.
- 7. Estimation of Vitamin C by 2,4,6-Dichlorophenol indophenol blue method.
- 8. Estimation of sodium by flame photometry.
- 9. Estimation of potassium by flame photometry

10. Isolation of oils by solvent extraction.

In addition, the following characterization studies were carried out:

- 1. Thin Layer chromatography (TLC) of isolated oils:
- The lipid fraction obtained after solvent extraction was subjected to separation by Thin Layer chromatography (TLC).
- The stationary phase utilized was silica gel, the mobile phase was a mixture of petroleum ether and diethyl ether (1:1) and iodine vapors were used as the developing agent.
- The resolved components were characterized by calculating their respective R_f values in the given chromatographic system.
- 2. Isolation of pigments in Asparagus and separation by Thin Layer chromatography (TLC)
- The aqeuos extract of asparagus was subjected to separation by Thin Layer chromatography (TLC).
- The stationary phase utilized was silica gel, the mobile phase was a mixture of petroleum ether and diethyl ether (1:1).
- The resolved components were characterized by calculating their respective $R_{\rm f}$ values in the given chromatographic system.

B) SENSORY EVALUATION:

Organoleptic analysis (sensory evaluation) is a scientific discipline that applies principle of experimental design and statistical analysis to the use of human senses (sight, smell, taste, touch and hearing) for the purpose of evaluating customer products. The discipline requires panels of human assessors on whom the products are tasted and recording the responses made by them. The overall quality of the food depends on the nutritional and other reading attributes. Thus while evaluating any food product it is necessary to assess its nutritive and sensory quality by applying statistical techniques to the results. It is possible to make inference and insights about the products under taste. Basic characteristics of an individual are inheritability and sensitivity. Trained panel evaluations are used to detect the oraganoleptic characteristics of food and non-food products. Customer test is an indicator of acceptability of food product. Sensory evaluation can be used for:

- Ingredients processing/packaging evaluation
- Shelf life testing
- Competitive comparisons
- Research applications
- The concept of sensory evaluation comprises of two aspects:
- Sensory evaluation with respect to manufacturer or product of the food materials.
- Sensory evaluation from the point of view of customer or acceptor of the food materials. A quality test panel has certain requirements before a sensory evaluation is carried out, which include the following:

- No consumption of tobacco/pan/food 2-3 hours before conduction evaluation.
- Good health and high degree of personal integrity.
- Average sensitivity.
- Intellectual curiosity and interest in sensory evaluation process.

Assessment of organoleptic acceptability of Asparagus pickle:

For this study, a brined and seasoned pickle of Asparagus was developed as the product for evaluation. This pickle aimed to be low-calorie, highly nutritive food, which elevates the natural flavor and biochemical benefits of Asparagus. In order to project the organoleptic appeal and acceptability of the developed Asparagus product, sensory evaluation was carried out with a semi-trained panel. A panel comprising of **35** panelists (biostatistically significant number of panelists) were chosen for evaluation.

Panelists were to answer a detailed questionnaire based on both non-invasive and invasive parameters. Non-invasive parameters assessed the general awareness of the consumer w.r.t. the various nutritional qualities of Asparagus, its incorporation into pickles and the acceptability of the idea of a pickle being made from Asparagus. In the invasive section of the questionnaire, panelists graded the samples based on various sensory attributes. It also focused on the marketability of the product, its value for money, consumer preferences for product consumption to elucidate the overall acceptability of Asparagus.



Asparagus Pickle

The data obtained from the questionnaires was evaluated using bio-statistical principles to ascertain the acceptability of the product. Also, the marketability of the product developed was ascertained using the parameter of Value for Money or VFM.

C) ASSESSMENT OF CRITICAL CONTROL POINTS (CCPs) IN THE PRODUCTION OF ASPARAGUS PICKLE: HACCP :

HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product. An efficient HACCP system aims to prevent hazards in production processes that can cause the finished product to be unsafe, and designs measurements to reduce these risks to a safe level. In this manner, HACCP is referred as the prevention of hazards rather than finished product inspection. The HACCP system can be used at all stages of a food chain, from food production and preparation processes including packaging and distribution.

Developing an efficient HACCP system basically helps in achieving consistent product quality by minimizing hazards that indirectly inculcate batch-to-batch variations in each level of manufacturing. Thus, the overall result of the implementation of the HACCP system helps achieves uncompromising quality.

Identification of Critical Control points:

A critical control point (CCP) is a point, step, or procedure in a food manufacturing process at which control can be applied and, as a result, a food safety hazard can be prevented, eliminated, or reduced to an acceptable level. In the case of novel product development, critical control points generally involve optimizing

culinary parameters for preparation of the recipe. In addition, synergistic parameters like hygiene and sanitation measures which indirectly influence product quality are also considered. The identification of critical control points basically revolves around defining the critical limits for any ambiguity in the manufacturing process. A critical limit is the maximum or minimum value to which a physical, biological, or chemical hazard must be controlled at a critical control point to prevent, eliminate, or reduce to an acceptable level. Monitoring activities are necessary to ensure that the process is under control at each critical control point. This is regularly achieved by quality testing of ingredients and routine sensory evaluation at various stages of product manufacturing.

One of the accessory requirements of a CCP system is the thorough understanding and implementation of corrective actions. These are actions to be taken when monitoring procedures indicate a deviation from an established critical limit. Corrective actions are intended to ensure that the product obtained is not injurious to health or otherwise adulterated as a result of the deviation. The ultimate aim of this procedure is to deliver a product with uncompromising quality to the consumer.



Process flowsheet for identification of CCPs for Asparagus pickle:

The CCPs for production of Asparagus pickle at various stages are elaborated as follows:

1. Raw ingredients:

- Look for firm, thin stems with deep green or purplish closed tips. The cut ends should not be too woody, although a little woodiness at the base prevents the stalk from drying out.
- Asparagus stalks should be rounded, and neither fat nor twisted. Asparagus should be used within 2 days after purchasing or else it will lose its flavor.
- Washing should be done using potable water. 2. *Pre-cooking process I:*
- The ice should be made from potable water.
- The container used should be properly washed and dried.
- Asparagus should be kept for exactly 10 minutes in ice water. 3. *Pre-cooking process II:*
- Bottle used for pickling should be made of glass of GRAS (generally regarded as safe) grade.
- Water used for sterilization should be potable.
- Boiling water should be used to clean the bottle.
- Pickling bottle should be completely dried before using. 4. Addition of condiments:
- Make sure that the bottle is completely dry.
- The dill seeds should be properly cleaned and free from any foreign material/adulterant.
- Garlic should be peeled and used in the specified quantity.
- Chilies should be washed and de-seeded.

- All ingredients to be used in the specified quantity.
- The equipment used for cutting, chopping, peeling etc. should be clean. 5. *Process of pickling:*
- Vinegar used should be food grade and be GRAS.
- Water used should be potable
- The utensil used for boiling should be clean.
- Exactly equal quantities of vinegar and water to be used.
- The mixture should be boiling and poured in hot conditions. 6. Addition on savory flavors:
- Salt and sugar should be used in specified quantity.
- Used iodized salt only
- Add salt and sugar only after the water and vinegar mixture is boiling. 7. *Harvesting of product:*
- 1/4th space on the top of the container should be left as headspace.
- The bottle should be sealed when the water and vinegar mixture is hot.
- Pickle should be allowed to mature for exactly 1 month.
- Do not open the lid before maturation is complete
- Keep in cool and dry place.
- Keep away from sunlight.
 - 8. Post- harvest technology:
- Once the bottle is opened it should be kept in clean and dry place.
- Use a clean spoon to take out the desired amount of the product.
- Do not use the same spoon again.
- Once opened use/consume the product within 1 month. 9. *Sensory evaluation:*
- The product should be presented in clean and dry container.
- The juice should be separated and only asparagus is to be served.
- Bread to be given as accompaniment.

D) SIMULATED PRODUCT DEVELOPMENT:

Convenience foods are usually developed in the industry using the technique of Simulated Product development. This is the advanced technique by which the manufacturer or food producer conceives a product in virtual space. The various attributes of the product are made concrete using out of the box thinking combined with the knowledge and expertise of food and nutritional sciences. Simulated Product development is the new innovation in product development because it is a more efficient technique of product development because it easily bridges gaps between the concept of the product and its translation into reality. It is more favored in the industrial scenario because it is a profitable venture. The costs associated with translating a virtual product into reality is much lower than developing tangible products with ultimately no market value.

The product is first evaluated by expert panels [usually Descriptive Flavor Assessment Panel (DFAP)] capable of conceptualizing and grading the product solely on the detailed description of its attributes. DFAP consists of individuals who are experts in Sensory evaluation techniques.

They are chosen to assess and constructively suggest constructive alterations to introduce further innovations in the product so as to make it a unique and tangible venture. The product can also be evaluated using this technique by utilizing panels of naïve consumers after thorough briefing to ascertain the mass acceptability, demand and market share of the conceived product even before it is translated into reality.

Product information:

Cream of asparagus soup is a soup prepared with asparagus, stock and milk or cream as primary ingredients. Ingredient variations exist, as do vegan versions. Cream of asparagus soup may be served hot or cold, and the soup may be finished with various garnishes such as chives, crème fraîche and sour cream.

Assessment of Consumer demand:

To assess the consumer demand and acceptability of the Asparagus soup, a simulated questionnaire was developed. The questionnaire focused on various non-invasive attributes of the simulated dip. After thorough briefing, the questionnaire developed was distributed to 35 random consumers. The data collected was analyzed bio statistically to ascertain consumer awareness, the attributes of the recipe which appeal the consumer and value for money (VFM) of the dip. The interpretation obtained was further used in product development.

E) ANTIMICROBIAL ACTIVITY:

The antimicrobial activity of Aqueous Asparagus Extract was assessed by well diffusion method against a set of food borne pathogens *E.coli, Bacillus licheniformis, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus cereus,* which were obtained from MTCC, India. The Muller Hinton agar plates were prepared and 100µl of each culture were added to individual plates and spread uniformly with an L-shape spreader. Wells of 6 mm diameter was made using a sterile cork borer and 20 µl of 10%w/v (Aqueous) Asparagus Extract was used for all strains except Pseudomonas aeruginosa which was treated with 30 µl of 10%w/v (Aqueous) Asparagus Extract. All extracts were added into individual wells and incubated at 37°C for 24 hrs and the Zone of Inhibition (ZOI) formed surrounding the well was noted.

IV. Data Analysis

A) SENSORY EVALUATION: i) Non-Invasive:

Panelist opinions on their frequency of consumption of Asparagus:



Panelist awareness on pickled vegetables:



Panelist opinions on whether the idea of pickled asparagus is appealing:



ii) Invasive:

Panelist opinions on whether they will buy Asparagus pickle if it were commercially available:



Panelist opinions on the pricing of Asparagus pickle (Rs 50 for 150g):



B) SIMULATED PRODUCT DEVELOPMENT:

Panelist awareness on the idea of Asparagus soup:



Panelist opinions on the idea of Asparagus soup:



Panelist opinions on the pricing of Asparagus soup:



Panelist opinions on whether they would buy Asparagus soup if it were commercially available:



V. Results

A) PROXIMATE ANALYSIS:

Nutrient	Amount in Raw asparagus (Per 100g)	Amount in Cooked asparagus (Per 100g)
Moisture	89.80g	93.82 g
Ash	0.608 g	0.304 g
Carbohydrates	0.054 g	0.073 g
Proteins	0.136 g	0.533 g
Vitamin C	3.50 mg	2.90 mg
Fiber	6.58 g	6.88 g
Calcium	0.120 g	0.112 g
Fat	3.20 g	2.90 g
Sodium	300 mg	181 mg
Potassium	375 mg	405 mg
Iron	0.725 mg	2.175 mg
Phosphorus	11.6 mg	61.6 mg



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The moisture content of asparagus is high and increases on cooking. Ash content decreases on cooking which is crude estimate that mineral content will also decrease on cooking. However, during the process of cooking there is loss in the amount of micronutrients.

Characterization studies:

i) TLC of lipids:

The results of the characterization studies of lipids by TLC is as follows:

Raw asparagus:

Component No	Solute front (cm)	Solvent Front (cm)	R _f value
1	0.6	4.4	0.136
2	1.0	4.4	0.277
3	2.4	4.4	0.545
4	2.6	4.4	0.590

Cooked Asparagus:

Component No	Solute front (cm)	Solvent Front (cm)	R_f value
1	0.6	4.4	0.136
2	1.2	4.4	0.272
3	1.6	4.4	0.303
4	2.0	4.4	0.681

ii) TLC of pigments:

The results of the characterization studies of lipids by TLC is as follows:



Raw asparagus:

Component No	Solute front (cm)	Solvent Front (cm)	R_f value
1	0.5	4.6	0.108
2	0.9	4.6	0.195
3	2.1	4.6	0.477
4	2.6	4.6	0.565
5	3.2	4.6	0.695
6	4.3	4.6	0.934

Cooked Asparagus:

Component No	Solute front (cm)	Solvent Front (cm)	R_f value
1	0.5	4.9	0.102
2	1.0	4.9	0.204
3	1.5	4.9	0.306
4	2.0	4.9	0.408
5	4.1	4.9	0.836

B) NOVEL PRODUCT DEVELOPMENT AND SENSORY EVALUATION:

- Asparagus is consumed infrequently be majority 77% of the panelists.
- Only 4% of panelists were aware of the idea of asparagus pickle.
- 94% of panelists agree that the idea of asparagus pickle is appealing.
- 92% panelists say that the pricing of asparagus pickle is appropriate.

• 92% panelists affirmed that they would buy asparagus pickle if it were marketed.

In the process of novel product development HACCP analysis was carried out and critical control points were established. Hazards were managed at every step in the product development that ensured that the product was of superior quality standards. The positive results of sensory evaluation of the product indicate that the HACCP was perfectly achieved. It follows that the product developed was exactly as desired by the panel and within all the norms specified under the critical control limits.

The trends in the result of sensory evaluation confirm that there is minimum awareness about the health benefits of consumption of asparagus. Consumption of asparagus is a matter of chance and not by choice, as determined by analysis of questionnaire. The sensory evaluation done by panelist exhibits very high organoleptic acceptance and high Value for Money (VFM) of the product developed from asparagus. An attempt to develop a convenience food using asparagus and assess the market need was made as part of this research. The analysis of data propagated the uniqueness of the product and showed high acceptability for the simulated product.

C) SIMULATED PRODUCT DEVELOPMENT:

- 86% of consumers are not aware about asparagus soup.
- 66% of consumers find the idea of asparagus soup appealing.
- 77% of the consumers find the asparagus soup to be appropriately priced.
- 94% consumers agree to buy the asparagus soup if it were marketed.

The call for convenience in food is the need of the hour. An attempt to develop a convenience food using asparagus and assess the market need was made. The data obtained was highly favorable since 94% of the consumers preferred to buy the product. This proves the fact that the product is innovative and the idea is liked by the consumers. This will help to create awareness about the nutritional benefits of asparagus as an unconventional ingredient.

D) ANTIMICROBIAL ACTIVITY:



The above photographic effects are of the plates of Standard microbial growth medium which depict the antimicrobial activity of the 10% w/v (Aqueous) Asparagus Extract.

Bacteria	Concentration of 10% aqueous	Volume of the extract	Zone of Inhibition diameter
	extract of Asparagus	(µL)	(in mm)
Pseudomonas aeruginosa	Neat	30	18.5 ± 0.70
Bacillus licheniformis	Neat	20	11.5 ± 0.71
Bacillus cereus	Neat	20	14.75 ± 0.5
E. coli	Neat	20	14.5 ± 0.70
Staphylococcus aureus	Neat	20	14.5 ± 0.71

The antimicrobial activity was present in the aqueous extract of Asparagus that confirms the efficacy of asparagus in combating pathogenic strains when consumed in diet. The results of the anti-microbial spectrum reconfirm its emphatic potential in a curative profile as a nutraceutical. This can be used to develop a formulation with holistic and sustainable benefits of health and wellbeing.

VI. Conclusion

Asparagus thus is a novel ingredient, which possesses the benefits of balanced nutrition, highly acceptable organoleptic profile and potential antimicrobial activity. Thus it exhibits great potential to be marketed in the form of innovative products in the food industry. In addition, mass manufacturing of asparagus products is feasible with the ideal identification of Critical control points. Lastly, the antimicrobial activity of Asparagus exhibits the untapped potential of marketing it as a potential nutraceutical to enhance human wellness.

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