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FLEXI-NOURISH HARNESSING NATURE'S ENZYMES, BRIDGING RESEARCH & NUTRITION FOR HOLISTIC HEALTH

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ABSTRACT

The development of an edible flaxseed-pineapple jelly is an innovative functional food of great nutritional and therapeutic interest. Flaxseed, with its high content of omega-3 fatty acids and dietary fibers, contributes significantly to the jelly's nutritional profile. Bromelain, a protease enzyme occurring naturally in pineapple, enhances its health benefits with anti-inflammatory, digestive, and antioxidant properties. The formulation aims to create a product that not only provides superior nutritional value but also preserves the bioactivity of its key constituents. This research investigates the formulation process, optimizing ingredient levels, and evaluating sensory properties. Results indicate that the inclusion of flaxseed and pineapple successfully maintains bromelain's bioactivity while enriching the overall nutrient content. These findings support the potential of this jelly as a functional food for digestive health and inflammation management, presenting a science- backed dietary choice for health-conscious consumers.

INTRODUCTION

Nutrient deficiencies, particularly of essential vitamins such as Vitamin C and Vitamin D, are prevalent worldwide, significantly impacting immune response, bone density, and overall

well-being. Functional foods fortified with bioactive compounds have emerged as effective dietary interventions to address these deficiencies. Flexi-Nourish, a ready-to- consume jelly, has been developed as a plant-based supplement incorporating bromelain-rich pineapple pulp, flaxseed, and agar to enhance its health benefits. Bromelain, a proteolytic enzyme derived from pineapple (*Ananas comosus*), is widely recognized for its therapeutic properties, including anti- inflammatory, antimicrobial, and antioxidant activities. Studies highlight bromelain's potent proteolytic activity, which aids digestion and improves nutrient bioavailability. Additionally, its role in modulating inflammatory pathways and immune responses makes it a valuable bioactive component in functional food development.

The formulation of this jelly aligns with the increasing demand for natural, plant-based nutritional supplements. By integrating the therapeutic properties of bromelain into a novel food matrix, Flexi-Nourish offers a convenient and sustainable dietary option suitable for individuals across all age groups. Further clinical studies can substantiate its long-term benefits in functional nutrition.

To assess its efficacy, Flexi-Nourish jelly undergoes comprehensive biochemical analyses. Antioxidant activity is measured using the DPPH assay, expected to demonstrate potent free radical scavenging activity due to the presence of Vitamin C and polyphenolic compounds (Sharma & Bhat, 2009). Antimicrobial properties are tested using the pour plate technique to determine total microbial count. Additionally, its anti- inflammatory effects are evaluated via the bovine serum albumin (BSA) denaturation assay, indicating its potential in managing inflammatory diseases (Mizushima & Kobayashi, 1968).

MATERIALS AND METHODOLOGY

- **Brief Overview of Jelly making process and its benefits**

The Pineapple jelly making process begins with cutting Pineapple in small pieces and homogenizing it in a blender for uniform mixture, later this pineapple pulp is cooked to gain thick consistency with sugar free gold and then strained. Next is preparation of flaxseed gel by boiling flaxseeds in hot water and then this gel is strained and collected for later use this procedure is followed by preparing Agar mixture from agar agar strips by soaking then in hot water and then cooking it till it dissolves, Final step is addition of these mixtures and stirred continuously till a uniform mixture is formed, lastly the jelly is kept a side to set at room temperature and stored in fridge for further use.

The ingredients used in making jelly provide various health benefits. Flaxseeds are rich in Omega-3 fatty acids, which help reduce inflammation, while their high fiber content supports digestion and gut health. They also aid in regulating cholesterol and blood sugar levels. Agar agar, a low-calorie, plant-based gelatin substitute, is an excellent source of fiber, promoting satiety and weight management. Additionally, it helps in blood sugar regulation and cholesterol control. Pineapple pulp is packed with bromelain, an enzyme known for its anti-inflammatory properties, and is high in Vitamin C, which boosts immunity and promotes skin health. It also supports digestion and bone health due to its manganese content. Sugar, when consumed in moderation, provides a quick source of energy, though excessive intake should be avoided. Lastly, water plays a crucial role in hydration, nutrient transport, and overall body function. Together, these ingredients contribute to digestive health, inflammation reduction, immunity support, and overall well-being when consumed in balanced amounts.

Fig 1. Pineapple pulp.

Fig 2. Preparation of jelly.

Fig. 3: Pineapple jelly.

The efficacy of Flexi-Nourish jelly is determined through three key biochemical assays.

1. Antioxidant Activity (DPPH Assay)

For the antioxidant assay, 2,2-diphenyl-1- picrylhydrazyl (DPPH) reagent was obtained, and ethanol (75% purity) was procured. Ascorbic acid, used as the standard antioxidant, was procured from Celin 500 Vitamin C tablet. The jelly samples were prepared using fresh pineapples (*Ananas comosus*), sourced from local market, and flaxseeds obtained from Organics. Absorbance measurements were carried out using a colorimeter purchased from Elico CL 223.

PROTOCOL

1. Preparation of Pineapple Jelly Extract

Weigh pineapple jelly according to 2% and 5% concentrations and make a fine paste in mortar and pestel and make volume up to 100 ml using ethanol in a conical flask.

I.e For preparation of 2% concentration of pineapple jelly extract dissolve 2 gram of jelly into 98 ml of ethanol and for 5% concentration of pineapple jelly extract dissolve 5 gram of jelly into 95 ml of ethanol.

2. Preparation of Standard Solution

For standard preparation of Ascorbic Acid a tablet of Celin 500 Vitamin C tablet was used. The tablet was crushed into fine powder using mortar and pestel and 100 mg was weighed and dissolved into 100 ml of ethanol in fraction to create stock solution.

3. Preparation of DPPH Reagent

To prepare the solution of DPPH reagent, 4mg of DPPH powder was weighed and dissolved into 100 ml of ethanol.

Table no. 1: Dilution Table for DPPH assay.

Std conc. (mcg/ml)	Stock solution (µL)	Ethanol (µL)	Final volume (µL)	DPPH reagent (µL)	Incubation for 30 minutes in dark	OD at 520 nm		
						I	II	III
10	10	990	1000	2000		0.82	0.74	0.63
40	40	960	1000	2000		0.50	0.52	0.50
100	100	900	1000	2000		0.03	0.04	0.10
2%	1000	-	1000	2000		0.03	0.03	0.02
5%	1000	-	1000	2000		0.04	0.03	0.03

4. The dilutions of stock solution was prepared at three concentrations ranging from low to high (low- 10 mcg/ml, medium – 40 mcg/ml and high – 100 mcg/ml).

Follow the given dilution table and perform the assay

5. Compare with Ascorbic acid (standard anti- oxidant drug). Ascorbic acid 100mg standard was prepared (100 mg - in 100ml ethanol) and measure the absorbance at 520nm.

2. Anti-Inflammatory Activity

Pineapple contains bioactive compounds such as bromelain, flavonoids, and polyphenols, which exhibit anti-inflammatory properties. Inflammation is often measured by analyzing the inhibition of proteins like BSA (bovine serum albumin) denaturation and changes in inflammatory cytokines such as IL-1β and IL-6. Using phosphate-buffered saline (PBS) ensures a stable pH environment, while BSA serves as a model protein for inflammation studies. The anti- inflammatory activity can be assessed using a BSA denaturation inhibition assay, where pineapple jelly extract is tested for its ability to prevent protein denaturation, mimicking in vivo anti-inflammatory effects. (Mans et all., 2022), (Ajibola et al., 2021).

Requirements

- Reagents & Chemicals

For Anti-inflammatory assay Phosphate-buffer saline (PBS) was obatined and Bovine serum albumin (BSA) was prepared, Pineapple jelly sample were prepared using fresh pineapple (*Ananas comosus*), sourced from local market, and flaxseeds obtained from Organics.) and extract was obtained by using water extraction metho. Diclofenac was used as positive control, Distilled water.

- Equipments used were Elico CL colorimeter at 640nm, Water bath at 70C, Micropipettes, Centrifuge Experimental Protocol Preparation of Pineapple Jelly Extract and Weigh 5 g of pineapple jelly and dissolve it in 50 mL of PBS. Then sonicate for 10 minutes and centrifuge at 5000 rpm for 10 minutes. Collect the supernatant as the test extract. For BSA Denaturation Inhibition Assay Prepare 0.2% BSA solution in PBS. (Breslin et al. 2021), Mix 1 mL of BSA solution with 100 µL of pineapple extract (varying concentrations: 50, 100, 150 µg/mL). And incubate at 37°C for 15 minutes and then heat at 70°C for 10 minutes. Cool and measure the absorbance at 640nm.

Compare with diclofenac (standard anti-inflammatory drug). Diclofenac 50mg standard was

prepared (1 tablet - in 100ml PBS) and measure the absorbance at 640nm.

3. Anti-Microbial Testing

The microbial load of the jelly sample was determined using 6-fold serial dilution and pour plate technique, which are widely implemented for the estimation of total microbial count. A 6-fold serial dilution was carried out to attain varying concentrations from the jelly sample.

Experimental Protocol

a. Sample Preparation and Serial Dilution

- 1 gram of jelly sample was initially diluted in 20 ml sterile saline solution (stock solution) and incubated for 30 mins.
- 1ml of stock solution or culture was transferred into the first tube (10^{-1}) i.e. 1ml into 9ml diluent for 1:10 dilution (v/v).
- It was then mixed thoroughly by repeatedly aspirating and dispensing the liquid within a pipette tip.
- 1ml from the first test tube was transferred to the second test tube containing 9ml of diluent (10^{-2} dilution). It was then mixed well and the process was continued through the desired number of dilutions.

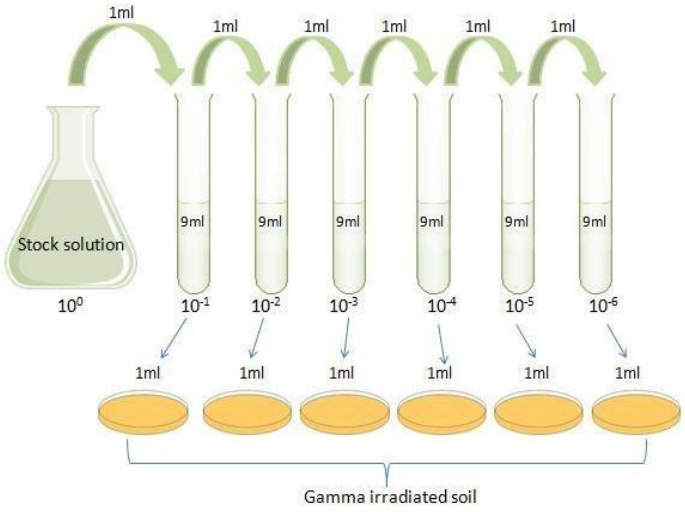

Fig No. 4: Dilution table – Schematic.

Table no. 5: Dilution table.

Tube	Sample	Diluent	Final Dilutions
1	1ml	9ml	10^{-1}
2	1ml	9ml	10^{-2}

3	1ml	9ml	10^{-3}
4	1ml	9ml	10^{-4}
5	1ml	9ml	10^{-5}
6	1ml	9ml	10^{-6}

b. Pour Plate technique Pour plate technique is used to ennumerate viable microorganisms in a sample by embedding them with a nutrient agar medium.

- Sterile Petri dishes with the corresponding dilution factors: are labelled.
- Using a sterile pipette and maintaining sterile conditions, 1ml of each dilution was transferred in 20ml molten nutrient agar present in the bumper tubes, mixed well and the contents of the tube were transferred to sterile Petri dish.
- Gently the plates were swirled in circular motion for even mixing and distribution of sample and agar and were allowed to solidified at room temperature.
- The process was repeated for all the dilutions 10^{-4},10^{-5}, 10^{-6}.
- Once solidified, the plates were kept inverted to prevent condensation and incubated at 37℃ for 24-48 hours.

4. Extraction and Quantification of Bromelain

FOR EXTRACTION

- Select pineapple core/stem
- Blend with Distilled water (1:2 w/v)
- Filter (Muslin Cloth/Whatmann filter paper)
- Centrifuge (15000 rpm, 5 minutes) (optional).
- Collect supernatant (Crude Bromelain Extract).
- Store at 4°C until use.

FOR QUANTIFICATION

- Prepare 1% gelatin solution.
- Take 5ml Gelatin in test tube.
- Add 1ml of Bromelain extract
- Incubate at 37°C for 30 minutes.
- Observe Clarity Change.
- Compare with Control (Without Bromelain).

RESULTS

1. Antioxidant Activity

Sample	Concentration (µg/mL or %)	Absorbance Values (Replicates)	Mean Absorbance	% DPPH Inhibition
Standard (Low)	10	0.82, 0.74, 0.63	**0.73**	**27.0%**
Standard (Medium)	40	0.50, 0.52, 0.50	**0.51**	**49.3%**
Standard (High)	100	0.03, 0.04, 0.10	**0.06**	**94.3%**
Pineapple Jelly	2%	0.03, 0.03, 0.02	**0.027**	**97.3%**
Pineapple Jelly	5%	0.04, 0.03, 0.03	**0.033**	**96.7%**

Table no. 6: Dilution Table for DPPH assay.

DPPH Inhibition Table

The mean absorbance of the standard concentrations and unknown concentrations (pineapple jelly) was calculated and applied in the following formula:

% Inhibition = [(A_control - A_sample) / A_control] × 100

According to the calculated percentage inhibition of DPPH, it was observed that the inhibition percentage was found to be high in 2% and 5% pineapple concentration samples, this indicates that the jelly possess high anti-oxidant properties.

2. Anti-Inflammatory Activity

- For pineapple jelly extract

Concentration	OD at 640nm
50mcg/ml	0.07
100mcg/ml	0.09
150mcg/ml	0.10

- For diclofenac standard

Concentration	OD at 640nm
50mg/100ml	0.43

- For pineapple jelly extract obtained using centrifugation (5g pineapple jelly in 50ml PBS)

Concentration	OD at 640nm
5gram	0.17

For anti-inflammatory assay, bromelain from pineapple extract was measured and diclofenac 50mg was used as standard for comparison. The pineapple jelly extract was prepared and measured at three different concentrations- 50mcg/ml, 100mcg/ml and 150mcg/ml. After

incubation at 37°C for 15 minutes and heating at 70°C for 10 minutes colorimetric analysis was performed at absorbance at 640nm, these readings were compared with standard (diclofenac) and anti-inflammatory activity was observed which can be due to bromelain present in pineapple.

Further, for detailed study Inhibition Percentage can be calculated by, **% Inhibition = [(A_control - A_sample) / A_control] × 100**

Higher inhibition means better anti-inflammatory activity.

Plot the Dose-Response Curve: Plot the % inhibition versus the concentration of the pineapple jelly extract to visualize the effectiveness. Determine the IC50 value (the concentration that inhibits 50% of BSA denaturation) to compare the anti-inflammatory potency of the pineapple extract with the standard anti-inflammatory agent.

3. Antimicrobial Activity

Fig No 5: PourPlate- 10^{-4},10^{-5},10^{-6}

Respectively

The pour plate technique was performed for detection of anti-microbial activity, after the incubation period of 24 hours it was observed that in the plates containing dilutions starting from 10^{-4}, 10^{-5}, and 10^{-6} minimal microbial growth was observed, the total microbial count of all three plates containing the dilutions was 1-2 isolated colonies; this indicates that the pineapple jelly might contain anti-microbial activity which inhibited the growth of micro-organisms.

4. Bromelain Extraction Results

The crude bromelain extract appeared light yellow to clear, indicating the presence of soluble proteins.

Yield varied based on pineapple part used (stem > core > fruit).

Gelatin Digestion Test Results:

Control (Gelatin + Water) remained cloudy, indicating no proteolysis. Bromelain-treated samples turned clear, showing gelatin breakdown due to enzymatic action.
Faster clearing = Higher enzyme activity.

CONCLUSION

This study highlights the potential of Flexi-Nourish, a pineapple bromelain and flaxseed-enriched jelly as a functional food with several benefits to health. The antioxidant analyses indicated that the jelly was a strong neutralizer of free radicals and might be able to guard against oxidative stress. The anti-inflammatory assay proved its capacity for protein denaturation inhibition, lending credence to its potential use against inflammation. Also, the antimicrobial analysis revealed that the jelly could have activity that restricts microbial growth and therefore contribute to food preservation as well as intestinal health.

The slow-firming process of bromelain maintained the bioactivity and guaranteed that the jelly still conveys its medicinal properties. This jelly, as a result of its nutrient-profile, may then be used as a natural dietary supplement derived from plants for health-conscious individuals intending to enhance their digestion, immune function, and general well-being. Additional scientific investigation, encompassing clinical experiments and consumer survey research, could ascertain its longer-term benefits as well as viability for use as an emerging technology functional food product.

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