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### Marine-Derived L-asparaginase as anti-Microbial and Food Preservative against *Listeria monocytogenes* isolated from Basa Fish Fillet (*Pangasius bocourti*)

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#### ABSTRACT

**M**arine environment is rich in many natural products with applications in different fields. L- asparaginase (L-asparagine amidohydrolase; E.C.3.5.1.1) is a widely distributed enzyme among terrestrial and marine organisms. It has an anticancer effect and it is used mainly for treatment of childhood Leukemia along with many other types of cancers. Another application of this enzyme is the mitigation of the carcinogenic acrylamide in baked and fried foods. A third application suggested in this article is its use as a food preservative due to its antimicrobial effect. In this work L- asparaginase purified from marine *Pseudomonas otitidis* EGY-NIOF- A1, accession number of OQ547301 is tested against *Listeria monocytogenes* isolated from Basa Fillet fish, identified and confirmed by using biochemical tests and MICROBACT *Listeria* 12L system. Amino acid analysis showed its rich content of Aspartic acid, glutamic acid, proline and arginine. The high content of these amino acids supports its antimicrobial effect. Future considerations should be devoted for the use of L- asparaginase as a food preservative. Biotechnological approaches for its production using low-cost strategies are recommended such as gene cloning strategy and genetic engineering.

#### INTRODUCTION

L-asparaginase (L-asparagine amidohydrolase; E.C.3.5.1.1) is an enzyme widely produced from many plants, microalgae and bacte-

ria. It is mainly used as anti-leukemic drug under the trade name of Kidrolase<sup>®</sup>, Oncaspar<sup>®</sup>, and L-asparaginase<sup>®</sup> (Van Trimont et al. 2022). It is also used under the trade name

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of " Acrylaways<sup>®</sup> " for acrylamide mitigation in fried and backed food (**Dourado 2020**). An additional application of such natural enzyme as antipathogenic potential for its antibacterial, antifungal activity making it a great candidate as a natural food grade preservative. The antimicrobial potential of L-asparaginase was reported against a wide range of gram-positive/gram-negative bacteria including (*Klebsiella pneumoniae*, *Listeria monocytogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*) (**Vimal and kumar 2022**).

Foodborne bacterial pathogens are the principal cause of product rejection and detention in the international seafood trade (**Ababouch et al. 2005**). *L. monocytogenes* contamination has a significant impact on seafood trade; According to FoodNet US, listeriosis was responsible for 30% of foodborne deaths from 1996 to 2005 and had a high case fatality rate of 16.9% (**Barton et al. 2011**). The minimal infectious dose for listeriosis is at least 100 CFU/g of food (**Ooi and Lorber 2005; Yang et al. 2007**). Infection can also be caused by a prolonged daily consumption of food containing 10-10<sup>5</sup> CFU/g of *L. monocytogenes* (**Maijala et al. 2001**).

The global proliferation of pathogens through seafood is a major hazard. The Rapid Alert System for Food and Feed (RASFF) in the EU has indicated that compared with other food product categories, seafood is second only to vegetables in the number of alerts activated between 2009 and 2012. Seafood import rejections by the RASFF comprised approximately 15% of the total product rejections in 2012 (**Anonymous 2013**).

Based on the number of such cases in the United States, EU, and Japan, foodborne bacterial pathogens are the principle cause of product rejection and detention in the international seafood trade (**Ababouch et al. 2005**).

*Listeria monocytogenes* has caused product detention in 4% of the cases recorded worldwide (**Huss et al. 2004**). Furthermore, contamination with *L. monocytogenes* has also led to product recalls (**Zhu et al. 2005**;

**Norhana et al. 2010**). Therefore, *L. monocytogenes* contamination has a significant impact on seafood trade; it causes direct and indirect financial losses because it necessitates sample reinspection as well as analysis and review of records, which can lead to product expiration and introduce costs associated with product recalls (**Norhana et al. 2010**).

The incidence of *L. monocytogenes* in fish products considered a subject of concern in the last few years especially in Basa Fish (*Pangasius bocourti*), a species of ray-finned fishes of family shark catfishes (**Hayat et al. 2018**)

The mechanism of anti-bacterial effect of L-asparaginase is suggested to be related to its hydrolytic action of the substrate, asparagine, responsible for cell proliferation that is upregulated and released during the pathogenic infection of streptolysin-secreting Gram-positive bacteria such as, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium botulinum*. In order to inhibit and delay the growth of both pathogenic and spoilage bacteria, chemical preservatives are used which have many antibacterial limitations. (**Baruch et al. 2014**).

The attention nowadays is devoted towards eco-friendly natural products as food grade preservatives against foodborne pathogens. In this study, the natural, eco-friendly, marine derived L- asparaginase is used as anti-listeria food preservative in Basa fillet.

L-asparaginase used in many domains including medical, industrial fields, as well as food technology. There are future plans to improve and develop the use of this biotechnology strategy on a large scale.

## MATERIAL and METHODS

### Screening of *Listeria monocytogenes* in Basa fillet

A total of 100 frozen Basa Fish Fillet were collected from different districts of Alexandria Governorate, Egypt for screening. The samples were put in sterile specimen bags under chilled conditions in icebox and taken to the

laboratory for analysis.

The isolation of *L. monocytogenes* was carried out according to (ISO 11290-1:2017 acc. to Elzbieta et al. 2020) by using primary and secondary selective enrichment broths (Half Fraser and Full Fraser Broth) by plating method. For each sample, 25 g was added to 225 mL of Half Fraser broth and stomached for 30 seconds. It was incubated at 30 °C for 24±2 hours. After 24±2 hours enrichment, the broth culture was agitated and 0.1 ml was transferred into a tube containing 10 ml full fraser broth, incubated at 37 °C ±1°C for 24±2 hours. Then a loopful was inoculated onto ALOA agar, incubated at 37 °C ±1°C for 24 – 48±2 hours). The plates were examined for blue-green colonies with white halos because of the presence of a chromogenic compound X-glucosidase in media which detects β-glucosidase which is present in all *Listeria* Spp, 3 suspected colonies were sub cultured on Tryptone Soya Agar (TSA, Oxoid) and incubated at 37 °C ±1°C for 24±2 hours. Phenotypic characterization including Gram's staining, catalase and motility tests were carried out according to the methods of (Hitchins and Jinne-man 2011).

#### Identification of *Listeria monocytogenes*:

For confirmation, five colonies presumed to be *L. monocytogenes* were taken from each from each suspected solid media. If any plate containing less than five presumed colonies, all the colonies were taken. The selected colonies were streaked onto the surface of pre-dried plates of TSYEA in a manner which allowed obtaining well-separated colonies

The plates were placed in the incubator set at 35°C or 37°C for 18 h to 24h. Typical colonies were 1 mm to 2 mm in diameter, convex, colorless and opaque with an entire edge. When the colonies were not well separated, a typical *Listeria* spp. colony was picked onto another TSYEA plate. Standard biochemical identification tests were carried out.

The following tests were carried out from colonies of a pure culture on the TSYEA. An isolated colony was taken and suspended in a

drop of hydrogen peroxide solution on a slide. gas bubbles were formed immediately which indicates a positive catalase reaction. The Gram stain was performed on a colony. Gram-positive slim and short rods were seen under light microscope. Using a straight loop, the motility agar was stabbed with a typical colony that was taken from TSYEA. Incubated for 48 h in the incubator set at 25 °C. Examined for growth around the stab. *Listeria* spp. was motile, with a typical umbrella-like growth.

Sheep blood agar plates were inoculated to determine the hemolytic reaction, incubated at 35 °C or 37 °C for 24 h ± 2 h. *L. monocytogenes* gave narrow, clear, light zones (β-hemolysis). Examine the plates in a bright light to compare test cultures with controls.

The carbohydrate utilization broths were inoculated with a culture from TSYEB. Incubated at 35 °C or 37 °C for up to 5 days. Positive reactions (acid formation) were indicated by a yellow color within 24 h to 48 h.

Confirmation of isolates was done by using MICROBACT *Listeria* 12L system according to (Amusan and Sanni 2018).

#### Isolation and Characterization of Marine derived L-asparaginase

The marine bacterium *Pseudomonas otitidis* EGY-NIOF-A1, accession number of OQ547301 was used for the production of L-asparaginase.

Protein quantification was done using Pierce™ BCA Protein Assay Kit Catalog Number 23225 (bicinchoninic acid assay kit, Thermo-Scientific) according to manufacturer's instructions, the method was firstly discovered by Smith et al. (1985).

Purified enzyme was used to test its antimicrobial activity against *L.monocytogenes*.

#### L-asparaginase Activity Assay

The Activity of the enzyme was estimated by determining the amount of released ammonia derived from hydrolysis of L-Asparagine by L-asparaginase according to a previously reported method using Nessler's reagent with slight modifications (Rasoul-Amini et al. 2014). Briefly, the enzyme assay reaction mix-

ture contained 900 L of 0.05 M L-Asparagine Substrate was dissolved in 0.05 M Tris-HCl buffer, pH 7.5, and (100 ul) of an appropriate dilution of the recombinant *aeruginosa* EGYII L-Asparaginase. This mixture was incubated at 37 °C for 2 min.

Then, (500 ul of 20% trichloroacetic acid (TCA) was added and the mixture was kept at room temperature for further 10 min. Then, (150 ul of this mixture was withdrawn and was mixed with (1400 ul) of distilled water. Nessler's reagent (200 ul) was added. After 10 min, the absorbance of developing color was measured spectrophotometrically at 450 nm. Control reactions were carried out as enzyme assay reactions except that TCA was added prior to enzyme sample addition. One arbitrary Unit of enzyme activity was defined as the amount of enzyme that releases 1 mole of ammonia per minute under the stated assay conditions. A standard curve using ammonium chloride was established. Specific activity was expressed in terms of U/mg (mole min<sup>-1</sup> mg<sup>-1</sup>). Protein determination was performed as previously reported using Coomassie Brilliant Blue G-250 and a standard curve of BSA was established (El-Sayed et al. 2012).

#### Amino acid analysis of Marine L-Asparaginase

Determination of amino acid composition was carried out on the purified enzyme using Sykam Amino Acid Analyzer (Sykam GmbH, Germany) according to the method of Speckman (mona et al. 2023). The instrument was equipped with Solvent Delivery System S 2100 (Quaternary pump with flow range 0.01 to 10.00 ml/min and maximum pressure up to 400 bar), Autosampler S 5200, Amino Acid Reaction Module S4300 (with built-in dual filter photometer between 440 and 570 nm with constant signal output and signal summary option) and Refrigerated Reagent Organizer S 4130.

Stock solution contains 18 amino acids (Aspartic acid, Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Tyro-

sine, Phenylalanine, Histadine, Lysine, Ammonia, Arginine) all amino acids concentrations are 2.5µMol/ml, except Cystine that was 1.25µMol/ml, then dilute 60 µl in 1.5 ml vial with sample dilution buffer then filtered using 0.22 µm syringe filter, then 100 µl was injected. Sample was pretreated with hexane and was allowed to macerate for 24 hours. Then filtered on Whatman no. 1 filter paper and the residue was transferred into a test tube where it was incubated in an oven with 5 mL 6N HCl for 24h at 110°C. After the incubation, the sample was then filtered using Whatman no. 1 filter paper, evaporated on rotary evaporator and dissolved completely in 200 ml dilution buffer. From this solution, the first dilution was prepared by diluting 4 mL to 50 mL dilution buffer, filtered using 0.22 µm syringe filter and 100 µl was injected according to the following parameters: LCA K06/Na column, gradient elution at 57°C-74°C with flow rate of 0.45 ml/min and wave length of 440 and 570 nm.

#### Antimicrobial activity of the marine enzyme using agar-well diffusion technique

In this method, Petri dishes were poured with a deep PYB agar medium previously inoculated with 1% (v/v) of an indicator bacterium. Several wells were punched out of the agar medium by using sterile cork borer (5mm in diameter). The base of each well was sealed with a drop of melted sterile water agar (20g agar per liter of H<sub>2</sub>O). Cell free supernatant of the strain that will show the highest antagonistic activity against the indicator strain was prepared by centrifuging the 24hrs old culture at 12,000 rpm for 10 min in a micro-centrifuge. A volume of 100µl was then transferred into each well, where the plates were incubated at 28°C (Heraeus incubator, Germany) for 24 hrs. After 24 hrs incubation, clear zones are supposed to appear around the wells indicating the inhibition of the indicator growth due to the antagonistic substance irradiated around each well. The radius of the inhibition zone (y) was measured in mm and the arbitrary unit (AU) for the clear zone was calculated as follows:

$$\begin{aligned} \text{(AU)} &= \pi y^2 \text{h (clear zone)} / \pi x^2 \text{h (well)} \\ &= y^2 \text{ (clear zone)} / x^2 \text{ (well)} \end{aligned}$$

Where ( $y^2$ ) is the square radius of the clear zone around each well, ( $x^2$ ) is the square of well's radius.

#### **Antimicrobial activity of the marine enzyme using fish fillet spiked samples with *L.monocytogenes*:**

Using six (6) samples; One sample as control negative, the second as control positive inoculated with  $10^2$  cfu/g of *L.monocytogenes* strain and the remained four samples were treated with different concentrations of L-asparaginase (442.5, 885, 1327.5, and 1770 U/ml) separately at the same time and they were contaminated with *L.monocytogenes* strain ( $10^2$  cfu/g). Spike sample was done by addition of *L.monocytogenes*  $10^2$  cfu to the initial suspension represented by 5 g of fish fillet and 45 ml of half Fraser broth. Then continue the procedure of ISO 11290-1: 2017 for isolation of *L.monocytogenes*. (Metekia and Ulusoy 2023).

#### **Sensory evaluation of fish fillet treated with L-asparaginase**

Purified marine L-asparaginase at the same four different concentrations (442.5, 885, 1327.5, and 1770 U/ml) were mixed with fish fillet samples. Sensory evaluation was carried out on the mixture by semi-trained panelists consisting of **9 persons**. The panelists were demanded their references for color, odor, texture, taste and overall acceptability attributes. The organoleptic assessment of the samples was performed at room temperature. The score for each item from the sensorial properties was conducted by 9-points descriptive scale (Hamad et al. 2022).

## **RESULTS**

### **Isolation of *Listeria monocytogenes* in examined fish samples:**

Out of 100 samples of frozen Basa Fillet fish, 10 isolates were identified as *Listeria monocytogenes* and one of them was chosen for further study.

### **Shape of *Listeria monocytogenes* colonies on Specific agar media:**

*Listeria* colonies showed a typical appearance on Oxford agar, greyish, encircled with a black zone. with a greenish reflection and with a black halo appearance. (Figure: 1).

On ALOA medium, *Listeria* spp. shows bluish green colored colonies. (Figure: 2).



Fig.1. Colonies suspected to be *Listeria* spp. cultured on Oxford agar  
Colonies appeared as gary, greenish colonies with black sunken centers



Fig. 2. Colonies suspected to be *L. monocytogenes* cultured on ALOA agar colonies appear as Bluish green colonies with hallow zone.



Fig. 3. Turbidity TSYEB indicates positive growth



Fig. 4. Positive fermentation of Rhamnose (yellow color) and negative for Xylose (violet color) that confirms presence of *L. monocytogenes*

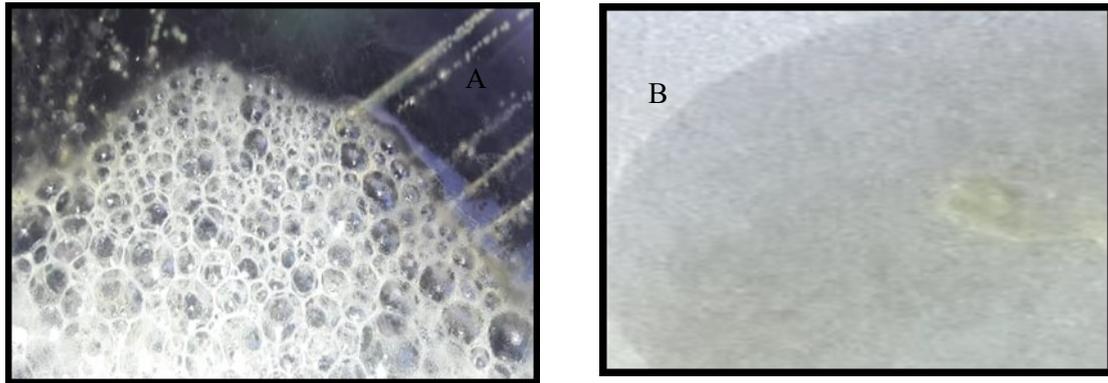


Fig.5. A. gas bubbles indicate Catalase Positive.

Fig. 5.B. absence of coloration indicates Oxidase negative reaction

**Microbact™ *Listeria* 12L system results:**

Colors and code obtained confirmed *L. monocytogenes* isolate by the resulted code 4-5-4-7 when compared with the predicted results in the Data Table provided in (Table.1).

([http://www.oxid.com/UK/blue/prod\\_detail/prod\\_detail.asp?pr=MB1128&..](http://www.oxid.com/UK/blue/prod_detail/prod_detail.asp?pr=MB1128&..))



Fig. 6. Microbact™ *Listeria* 12L system for *L.monocytogenes*

**Characterization of marine L-asparaginase**

Purified marine *Pseudomonas otitidis* EGY-NIOF-A1 L-asparaginase activity was measured as mentioned in before and found to be 4425 U/ml.

The protein concentration of the purified enzyme was **3.587 µg/µl**. The amino acid composition in (Table 1) and Fig. (7) Shows its amino acid contents that was rich in, proline (5.38), aspartic acid (3.9) and arginine (3.15) mg/100 mg

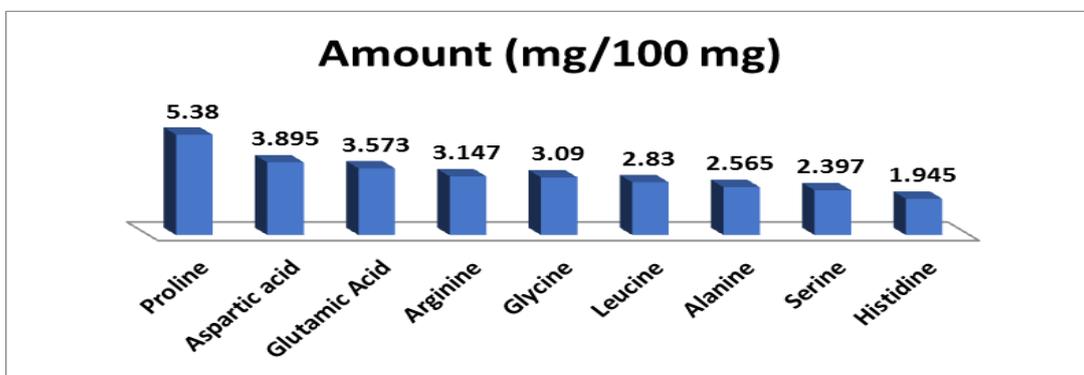


Fig. 7. Chart demonstrating Amino acid composition of purified marine *Pseudomonas otitidis* EGY-NIOF-A1 L-asparaginase

Table 1. Amino acid composition of purified marine *Pseudomonas otitidis* EGY-NIOF-A1 L-asparaginase

amino acid	Amount (mg/100 mg)
Aspartic acid	3.895
Threonine	0.918
Serine	2.397
Glutamic Acid	3.573
Proline	5.38
Glycine	3.09
Alanine	2.565
Valine	1.794
Methionine	0.384
Isoleucine	0.809
Leucine	2.83
Tyrosine	1.244
Phenylalanine	1.666
Histidine	1.945
Lysine	1.749
Arginine	3.147

#### Antimicrobial effect against *L. monocytogenes*

The antimicrobial potency of Purified marine *Pseudomonas otitidis* EGY-NIOF-A1 L-asparaginase was tested against *L. monocytogenes* isolated from Basa fish fillet (*Pangasius bocourti*) using agar well diffusion method. A clear inhibition zone was obtained compared to 10 mg/ml of Gentamycin antibiotic as a positive control (Figure 8).



Fig. 8. Anti-microbial effect of Purified marine *Pseudomonas otitidis* EGY-NIOF-A1 L-asparaginase against *L. monocytogenes* isolated from Basa Fillet Fish.

#### Antimicrobial activity of the marine enzyme using fish fillet spiked samples with *L.monocytogenes*:

*L. monocytogenes* was isolated from positive control sample (without addition of L-asparaginase and not isolated from the treated samples which were treated with four concen-

trations of L-asparaginase and also not isolated from the control negative (Blank) sample (Table, 2). These results confirmed that L-asparaginase has good antimicrobial effect against *L. monocytogenes*.

Table 2. Efficiency of of L-asparaginase on treated fish matrix

Samples under test		Treated Spiked Samples			
Control Negative (Blank Sample)	Control Positive Spiked Sample with $10^2$ cfu/g <i>L.monocytogenes</i>	442.5 U/ml	885 U/ml	1327.5 U/ml	1770 U/ml
Negative isolation of <i>L.monocytogenes</i>	Positive isolation of <i>L.monocytogenes</i>	ND	ND	ND	ND

ND= Not Detected (< 10 cfu/gm)

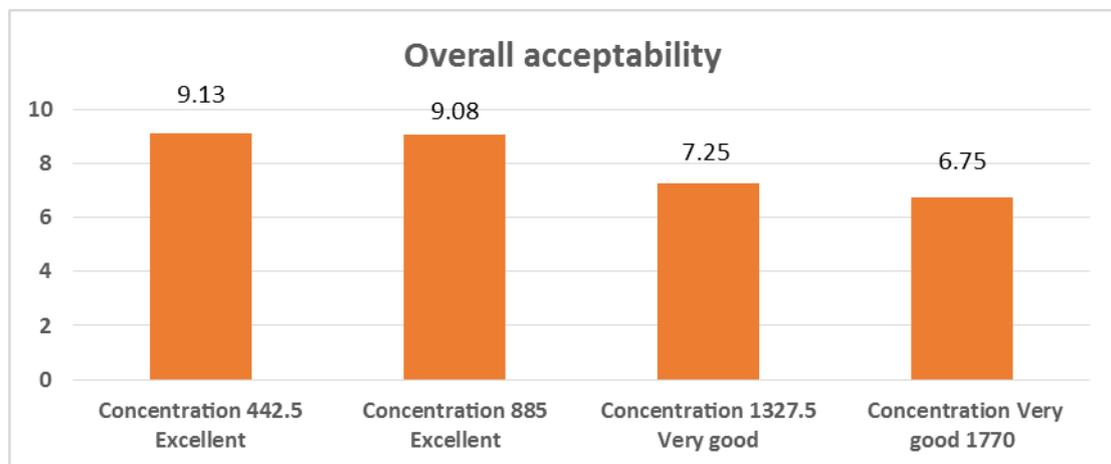
### Sensory evaluation of fish fillet treated with L-asparaginase

Fish fillet samples were mixed with various ratios of L. asparaginase and the sensory properties were evaluated. As shown in (Table 3). The sensory attributes of treated fish fillet samples scored excellent at the lowest concentra-

tions (442.5 – 885 U/ml L-asparaginase). At concentration of 1327.5 and 1770 U/ml, sensory properties scored very good. These results indicate the valuable effect of L. asparaginase on the sensory properties of fish fillet in addition to its antimicrobial and antioxidant effects.

Table 3. Sensory attributes of fish fillet samples prepared with different concentrations of *L. asparaginase*

Sensory parameter	Sensory scores & Concentration (U/ml)			
	442.5	885	1327.5	1770
color	9	9	7	6
odor	9	8	7	6
Texture	9	9	6	6
Taste	8.5	8	7	7
Mean overall acceptability	9.13	9.08	7.25	6.75

Fig.9. Mean overall acceptability of fish fillet after addition of *L. asparaginase* at different concentrations

## DISCUSSION

*Listeria monocytogenes* is a foodborne pathogen that can cause listeriosis, a serious illness that can be fatal, especially in pregnant women, newborns, and people with weakened immune systems. Basa fish, a type of catfish that is often farmed in Southeast Asia, has been linked to several listeriosis outbreaks in recent years ( **Kousta et al. 2011**).

*Listeria* can live in a wide range of temperatures, including those that are found in refrigerators. This makes it difficult to control, and it can survive even after food has been cooked. Basa fish is often imported frozen, which can increase the risk of *listeria* contamination ( **Osek et al. 2022**).

In 2016, Listeriosis linked to Basa fish was responsible for many illnesses and deaths in the United States. This was traced back to a single farm in Vietnam, and it was believed that the fish had become contaminated during the harvesting or processing. In 2018, another occurrence of listeriosis linked to Basa fish was reported in Canada resulting in many illnesses and deaths. This was traced back to a single supplier of Basa fish, and it was believed that the fish had become contaminated during the transportation or storage process. These outbreaks highlighted the importance of taking steps to prevent *listeria* contamination of Basa fish. Many chemical preservatives are used to inhibit food born bacterial growth mainly by interfering with the metabolism of bacteria making them difficult to grow. From these chemical preservatives: 1) Sorbic acid and benzoic acid, these preservatives are used in a variety of foods, including cheese, wine, baked goods, and fruit juices ( **Chan 2015**). 2) Propionic acid: usually used in baked goods, such as bread and cakes. 3) Sulfites, these preservatives are used to prevent the browning of fruits and vegetables as well as preventing the growth of bacteria in some foods. However, sulfites can cause allergic reactions in some people, so they are not always used in fish. And 4) Lactic acid: produced by lactic acid bacteria ( **Reddy 2014**).

It is worth to mention that not all chemical preservatives are safe for everyone. Some people may be allergic to certain preservatives. The best alternative is natural preservatives including L-asparaginase.

L-asparaginase catalyses the deamination of L-asparagine, an amino acid that is essential for bacterial growth. This makes L-asparaginase a potential antibacterial agent ( **Vimal and kumar 2021**). The amino acid content of the purified marine *Pseudomonas otitidis* L-asparaginase showed its enrichment mainly with the following amino acids. Aspartic acid, glutamic acid, proline and arginine, these four amino acids have been shown to have antimicrobial effects. The high negative charge of aspartic and glutamic acids can disrupt the cell membrane of bacteria by creating pores. Proline is a non-polar amino acid that can interfere with the folding of bacterial proteins. Arginine is a basic amino acid that can chelate metal ions, which are essential for bacterial growth ( **Freeman 1963; Kumar and sharma 2016**).

The antimicrobial effects of these amino acids have been explored in a number of different applications. For example, they have been used to develop new antimicrobial peptides and to improve the efficacy of existing antibiotics. They have also been studied as potential food preservatives and as treatments for bacterial infections ( **Zhang et al. 2019**).

The results of application of L.asparaginase on fish matrix contaminated with *L.monocytogenes* demonstrated that L.asparaginase has strong antibacterial effect on *L.monocytogenes* which resulted in complete decontamination of experimentally spiked *L.monocytogenes* by 100 cfu/g (four successful trials) which is agreed with **vimal and kumar 2018** , they demonstrated that infections caused by *L.monocytogenes* can be treated by L-asparaginase.

Treated Basa fillet with marine L-asparaginase as a preservative was tested for its sensory effect and overall acceptability was

found to be very good to excellent and also has good antimicrobial effect on *L.monocytogenes* growth. Our findings are in parallel with those reported by **Vimal and Kumar (2021)**, the authors concluded that *L. asparaginase* was explored as an anti-infectious agent. The results of the present study provide critical information for understanding the potential role of *L. asparaginase* as an antimicrobial and antioxidant candidate to produce a healthy fish fillet, low/free of *L. monocytogenes*, with good organoleptic properties.

The results of application of L-asparaginase on fish matrix contaminated with *L.monocytogenes* demonstrated that L-asparaginase has strong antibacterial effect on *L.monocytogenes* which agreed with **vimal and kumar (2018)**. The outcomes of this study will lead future direction of using of *L-asparaginase* in fish fillet production.

From this concept, many studies tested its anti-microbial potency using either the free form of the enzyme, or the immobilized form, or even the enzyme purified from different bacterial or fungal sources, even the recombinant form. As an example, a study evaluated both the free and immobilized L-asparaginase using chitosan nanoparticles and found that L-asparaginase was more effective against *Listeria monocytogenes*, the common food pathogen, compared to the antibiotic ampicillin (**Zhang 2018**). There are many examples of the use of L-asparaginase as a food preservative. For instance, a study by **Chen et al. (2019)** found that L-asparaginase could inhibit the growth of spoilage bacteria including *Escherichia coli* and *Pseudomonas aeruginosa* in fresh-cut vegetables thus increase its shelf-life.

Another study carried by **Li et al. (2020)** who found that L-asparaginase could be used to improve the safety of milk. The study found that L-asparaginase could inhibit the growth of *Listeria monocytogenes* in milk, even at low concentrations.

The use of **L-asparaginase** as a promising food preservative has many advantages; First, It is a natural enzyme, so it is relatively non-toxic and could be extracted from many differ-

ent plant, fungal and bacterial sources either marine or terrestrial. All the results of these studies suggest that L-asparaginase has the potential to be a safe and effective food preservative. L-asparaginase has been granted Generally Recognized as Safe (GRAS) status by the U.S. Food and Drug Administration (FDA). This means that the FDA has determined that L-asparaginase is safe for use in food when used according to good manufacturing practices (**Cachumba et al.2016**). L-asparaginase has been used in food processing to reduce the carcinogenic acrylamide content in baked good and fried potato for over 20 years without any reported safety concerns. It is currently used in a variety of food products, including bread, baked goods, and breakfast cereals (**Hendriksen et al. 2009**). All the results of these studies suggest that L-asparaginase has the potential to be a safe and effective food preservative. However, there are also some challenges to the use of L-asparaginase as a food preservative.

One challenge is that L-asparaginase can be inactivated by some food ingredients, such as ascorbic acid. This can be neglected in foods without such ingredient.

Another challenge is that L-asparaginase can be expensive to produce; this can be overcome by using cheap, eco-friendly methods, especially biotechnological approaches for its production such as gene cloning strategy and genetic engineering. As an example, L-asparaginase gene was isolated from marine *Pseudomonas aeruginosa* and was successfully expressed in *E. coli* cells using gene cloning strategy (**Saeed et al. 2018**).

In addition to recombinant DNA technology, There are a number of different methods for the production of L-asparaginase, including: 1) Submerged fermentation (SMF): This is the most common method for producing ASNase from *Erwinia chrysanthemi*. It involves growing the producing organism in a liquid medium under controlled conditions. SMF is a scalable process that can produce large quantities of ASNase. However, it can be a relatively expensive process, and it can gen-

erate large amounts of wastewater. The other method is: 2) Solid-state fermentation (SSF): This method involves growing the producing organism, e.g. *Aspergillus niger*, *Serratia marcescens*, on a solid substrate, such as bran or wheat straw. SSF is a less expensive process than SMF, and it generates less wastewater. However, it is a less scalable process, and it can be more difficult to control the fermentation conditions (Abdel-Fattah and Olama, 2002; Ghosh et al. 2013)

## CONCLUSION

**L**-asparaginase is widely distributed enzyme with many different applications. Marine *Pseudomonas otitidis* EGY-NIOF-A1, has a good anti-microbial effect against *Listeria monocytogenes*. The amino acid content of this enzyme suggests its anti-listerial growth. Other forms of L-asparaginases purified from other sources may differ in its composition and may show antagonistic effect towards other food pathogens. It is recommended to search for other forms and sources of L-asparaginases from other marine or terrestrial sources and examine its effect against many other pathogens in an approach to use it as a natural food preservative. Gene cloning strategy and genetic engineering could be devoted for its low-cost and large scale production.

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