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Effect of Packaging Materials on the Microbiological Quality and Shelf Life of Tuna Bone Flour-Fortified Pumpkin Noodles

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ABSTRACT

This study aimed to evaluate how different packaging types affect the quality of yellow pumpkin noodles fortified with tuna bone flour. Although pumpkin noodles fortified with tuna bone flour have improved nutritional value, limited information is available on how packaging materials affect their microbiological stability and shelf-life prediction during storage. The experiment was conducted using a Completely Randomized Design (CRD) with five treatments and three replications. Data were analyzed using analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DNMRT) at a significance level of $\alpha = 1\%$. Shelf life was estimated using the critical moisture content approach based on moisture increase during storage. The results showed that the types of packaging (PP, PE, PS, aluminum foil, and paper) had a highly significant effect on moisture content, Total Plate Count (TPC), and mold count of pumpkin noodles. The values of moisture content, Total Plate Count (TPC), mold count, and *Escherichia coli* met the requirements of SNI 8217:2015, while *Salmonella* sp. met the food safety requirements of SNI 7388:2009 regarding the maximum limit of microbial contamination in food. Pumpkin noodles stored in aluminum foil packaging showed the best quality and longer shelf life. On the 9th day of storage, the product had a moisture content of 9.64%, Total Plate Count (TPC) of 6.9×10^3 CFU/g, *Escherichia coli* <3 MPN/g, mold count of 2.5×10^3 CFU/g, and *Salmonella* sp. negative/25 g. Based on the predictive model used in this study, aluminum foil packaging was estimated to provide the longest shelf life. However, this prediction requires validation through long-term storage experiments.

Contribution to Sustainable Development Goals (SDGs):

SDG 2: Zero Hunger

SDG 3: Good Health and Well-Being

SDG 12: Responsible Consumption and Production

1. INTRODUCTION

1.1. Research Background

Noodles are a common food item consumed by the public, alongside rice, a staple food. Noodles come in four types: fresh, wet, dry, and instant. Dry noodles are fresh noodles dried to 8-10% moisture content [1]. The raw material for dry noodles is wheat flour, which is currently imported, and efforts are

underway to reduce its use. Adding fiber to noodle products requires the use of wheat substitutes such as sweet potato, cassava, and pumpkin. Yellow pumpkin (*Cucurbita moschata* Duch.) is widely cultivated due to its high productivity, ease of cultivation, and abundant availability [2]. Pumpkin has advantages such as high carbohydrate, vitamin, and fiber content, making it a substitute for wheat flour and a natural coloring agent for dry noodles.

Fish bones are a by-product of various types of fish fillets, especially those whose calcium content can be utilized. Tuna fish



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bones contain a lot of calcium, phosphate, magnesium, sodium and fluoride [3]. Tuna bone meal contains higher calcium than other fish meal. Catfish bone meal contains 13.48% calcium, yellowfin tuna bone meal 2.12% calcium, patin bone meal 30.95% calcium, and tuna bone meal ranges from 39-40% [4]. To maintain the quality of pumpkin noodles, preservation is necessary, one way of doing this is through packaging.

Packaging plays a crucial role in maintaining product quality and enhancing marketability. A good product should also have good packaging; besides maintaining quality, packaging can also attract consumers to buy the product [5]. Product packaging is the wrapping of a product. Products have a specific shelf life, influenced by several factors, including microbial growth. Microbes that can grow on food products include bacteria, fungi, and mold [6].

Microorganisms are microscopic living organisms capable of growing in food products under favorable environmental conditions [7]. To determine the extent of food spoilage, microbiological testing is necessary. The purpose of this study was to determine the effect of various packaging on the quality of dried pumpkin noodles fortified with tuna fish bone meal. The benefits of this study are to provide information on the best packaging for dried pumpkin noodles fortified with tuna fish bone meal

1.2. Literature Review

Dried noodles are one of the most widely consumed wheat-based food products in Indonesia and serve as an important alternative staple after rice. This product generally has a relatively low moisture content (approximately 8–10%), which contributes to a longer shelf life compared to fresh or wet noodles [8]. However, dried noodles can still deteriorate during storage, including moisture absorption, textural changes, and damage from microbial activity and chemical reactions. The stability of dried noodles is influenced by several factors, including raw material composition, particularly protein and starch content, as well as storage conditions and packaging systems [9].

Wheat flour, as the primary raw material in noodle production, plays a crucial role in determining product characteristics, mainly through its gluten content, which contributes to elasticity and chewiness. High-protein wheat flour is generally preferred to produce noodles with better texture and structural integrity. However, current developments in noodle products are not only focused on textural quality but also on improving nutritional value through the incorporation of alternative ingredients or fortification [10].

One potential ingredient for substitution is pumpkin (*Cucurbita moschata*), which is rich in β -carotene, vitamins, minerals, and dietary fiber. The β -carotene content in pumpkin acts as provitamin A and an antioxidant beneficial for human health. The incorporation of pumpkin into noodle products not only enhances functional value but also provides an attractive natural color. Nevertheless, pumpkin's chemical composition, particularly its moisture and sugar content, may influence the physical properties and storage stability of the final product [11].

In addition, fortifying with tuna fish bone flour is a promising approach to enhance nutritional value, especially as a source of calcium. Tuna fish bones are known to contain high levels of minerals, particularly calcium and phosphorus, as well as collagen protein. The utilization of fish bone waste into flour not only increases its economic value but also supports sustainable

waste management in the agro-industrial sector. However, the incorporation of fish-based materials may increase the risk of lipid oxidation and undesirable odors, which can negatively affect product shelf life [12].

In this context, packaging plays a significant role in maintaining food quality during storage. Packaging functions as a barrier against external factors such as moisture, oxygen, light, and microbial contamination [13]. Various packaging materials, such as plastics (polypropylene and polyethylene) and aluminum foil, exhibit different permeability characteristics toward water vapor and oxygen. Therefore, the selection of appropriate packaging materials is essential to control quality deterioration, particularly in dried products that are sensitive to moisture absorption and oxidation [14].

Besides physical and chemical factors, microbiological aspects are also critical parameters in determining food shelf life [15]. Food products can act as substrates for microbial growth, leading to spoilage and potential health risks. Therefore, microbiological analyses such as total plate count, yeast and mold count, and detection of pathogenic bacteria are necessary to ensure product safety and quality during storage [16].

Although previous studies have investigated pumpkin-based noodles, tuna bone flour fortification, and the role of packaging materials in food preservation, they have generally examined these aspects separately. Limited information is available regarding the comparative performance of different conventional packaging materials in maintaining the microbiological quality and predicting the shelf life of pumpkin noodles fortified with tuna bone flour during storage. Furthermore, most previous studies have focused primarily on nutritional composition, physicochemical characteristics, or sensory quality, while comprehensive evaluation of microbiological stability during storage remains limited. Therefore, this study provides new information regarding the comparative effectiveness of five commonly used packaging materials, namely polypropylene (PP), polyethylene (PE), polystyrene (PS), aluminum foil, and paper, in maintaining the microbiological quality and estimating the shelf life of tuna bone flour-fortified pumpkin noodles. The findings are expected to contribute to the selection of appropriate packaging materials for fortified dried noodle products with improved storage stability.

Fish bone flour has recently gained considerable attention as a functional food ingredient because of its high calcium, phosphorus, collagen, and bioavailable mineral contents. In addition to improving the nutritional value of food products, the utilization of fish bone flour also supports the sustainable valorization of fish processing by-products and contributes to the circular economy. Recent studies have shown that fish bone flour can be successfully incorporated into dried noodle formulations, increasing calcium content while maintaining acceptable physicochemical and sensory characteristics at appropriate concentrations. Nevertheless, excessive substitution may alter dough rheology, microstructure, texture, and cooking quality, indicating that optimization of formulation and post-processing conditions remains essential [21].

Shelf life is influenced not only by microbiological deterioration but also by physicochemical changes such as moisture uptake, water activity, texture degradation, lipid oxidation, and color changes. Therefore, microbiological analysis alone provides only a partial assessment of product stability.

1.3. Research Objective

This study aims to determine the effect of various packaging types on the quality and shelf life of yellow pumpkin noodles fortified with tuna bone meal.

2. MATERIALS AND METHODS

This study was conducted using a Completely Randomized Design (CRD) consisting of five treatments and three replications. The treatments involved different types of packaging for pumpkin noodles fortified with tuna bone flour, namely polypropylene (PP), polyethylene (PE), polystyrene (PS), aluminum foil, and paper packaging.

Table 1. Formulation of yellow pumpkin noodles supplemented with tuna fish bone flour using different packaging materials

Ingredients	Unit	A	B	C	D	E
Wheat flour	g	70	70	70	70	70
Yellow pumpkin puree	g	30	30	30	30	30
Tuna fish bone flour	g	2	2	2	2	2
Water	mL	30	30	30	30	30
Egg yolk	g	10	10	10	10	10
Packaging material	–	PP	PE	PS	Aluminum foil	Paper

2.1. Research Objective

This study aims to analyze the factors of rice production and the relationship of subsidized fertilizer in Madang Suku I District of East OKU Regency.

3. RESULT AND DISCUSSION

3.1. Water Content Analysis

The analysis of variance results showed that different packaging types had a highly significant effect ($P < 0.01$) on the water content of pumpkin noodles. The average water content of pumpkin noodles in various packaging is presented in Table 2:

Table 2. Average water content of various packages of pumpkin noodles

Packaging types	Water content (%)	
	Day-0	Day-9
A (PP)	9.11	10.65±0.17 b
B (PE)	9.16	11.37±0.51 bc
C (PS)	9.18	11.99±0.57 cd
D (Aluminium Foil)	9.16	9.64±0.46 a
E (Paper)	9.11	12.6±0.62 d

Note: Mean values followed by different letters indicate significant differences ($P < 0.01$)

Table 2 shows the moisture content of pumpkin noodles with added tuna bone meal, packaged in various packaging. The highest moisture content was found in paper-packaged pumpkin noodles at 12.65%, while the lowest moisture content was found in aluminum foil-packaged noodles at 9.64%. The water content of pumpkin noodles using aluminum foil packaging showed the lowest increase compared to other packaging. The increase in

moisture content during storage was attributed to moisture migration driven by differences in water vapor pressure between the product and the surrounding environment. Moisture adsorption occurred when water vapor from the surrounding atmosphere diffused through the packaging material and was absorbed by the dried noodles. The rate of moisture transfer depends largely on the water vapor transmission rate (WVTR) of the packaging material. Aluminum foil possesses an extremely low WVTR and excellent barrier properties against water vapor diffusion, thereby minimizing moisture uptake and maintaining product stability throughout storage. In contrast, paper packaging exhibits high water vapor permeability, allowing greater moisture adsorption and resulting in a higher increase in moisture content.

This is because aluminium foil packaging is waterproof and airtight, prevents direct contact with air, and produces the lowest water vapour in pumpkin noodles compared with other packaging. This protects the product inside from gas or water exchange with the outside during storage. Aluminum foil packaging is part of modified atmosphere packaging (PAT), namely product packaging that has adequate protection against light, moisture and oxygen, is waterproof, inhibits the entry and exit of gases, thereby reducing microbial growth, reducing damage by enzymes, and extending shelf life [13].

According to Putri (2016) states that the permeability of each package is different and will affect the rate of water vapour transmission; the lower the rate of water vapour transmission of a package, the less water vapour is able to penetrate the package [11]. The highest water content was found in pumpkin noodles with paper packaging at 12.65%, while the lowest water content was found in pumpkin noodles with aluminum foil packaging at 9.64%. The water content obtained in this study met the requirements for dry noodles SNI 8217-2015 maximum 13%.

3.2. Total Plate Count (TPC)

The diversity analysis results show that packaging type has a highly significant effect ($P < 0.01$) on the Total Plate Count (TPC) of pumpkin noodles. Table 3 presents the average Total Plate Count (TPC) of pumpkin noodles across packaging types.

Table 3. Average Total Plate Count (TPC) on pumpkin noodles

Packaging types	TPC (CFU/g)	TPC (CFU/g)
	Day-0	Day-9
A (PP)	6.4x10 ³	9.1x10 ³ a
B (PE)	6.9x10 ³	6.2x10 ⁴ b
C (PS)	6.8x10 ³	9.5x10 ⁴ c
D (Aluminium Foil)	6.1x10 ³	6.9x10 ³ a
E (Paper)	6.3x10 ³	1.8x10 ⁶ d

Note: Mean values followed by different letters indicate significant differences ($P < 0.01$)

Table 3 shows that the Total Plate Count (TPC) in pumpkin noodles in various packages on day 0 ranged from 6.1x10³ – 6.9x10³ CFU/g while the Total Plate Count (TPC) on day 9 ranged from 6.9x10³- 1.8x10⁶ CFU/g. Based on the DNMRT further test at the 1% level, each treatment showed a very significant difference in the Total Plate Count (TPC) of pumpkin noodles in aluminum foil packaging had a low Total Plate Count (TPC) of 6.9 x 10³ while pumpkin noodles in paper packaging had a high Total Plate Count (TPC) of 1.8 x 10⁶. Total Plate Count (TPC) in paper packaging showed the greatest increase compared

with other packaging. There are differences Total Plate Count (TPC) pumpkin noodles are affected by the different packaging they're stored in. Differences in microbial populations among packaging materials can be explained by their oxygen barrier properties. Oxygen permeability plays a crucial role in determining the availability of oxygen inside the package, which directly influences the growth of aerobic microorganisms. Packaging materials with low oxygen transmission rates (OTR), such as aluminum foil, effectively restrict oxygen diffusion into the package, thereby limiting aerobic microbial metabolism and reducing bacterial proliferation during storage. Conversely, packaging materials with higher oxygen permeability, such as paper, allow greater oxygen penetration, creating favorable conditions for microbial respiration and cell multiplication.

Paper packaging generally exhibits higher oxygen and water vapor permeability than polymeric and aluminum-based packaging materials. The higher permeability allows oxygen and moisture from the surrounding environment to diffuse into the package, creating favorable conditions for aerobic bacterial growth and microbial metabolism. As microorganisms utilize available oxygen and nutrients for cellular respiration, their population increases during storage, resulting in higher Total Plate Count (TPC) values. In contrast, aluminum foil acts as an excellent barrier against oxygen and water vapor, thereby inhibiting microbial proliferation and preserving the microbiological quality of the product.

Furthermore, moisture uptake during storage contributes to an increase in water activity (a_w), which is one of the most important factors controlling microbial growth. Packaging materials with poor moisture barrier properties permit greater water vapor adsorption, increasing water activity and creating a more favorable environment for bacterial proliferation. This finding is consistent with the lower moisture content observed in aluminum foil packaging, which also exhibited the lowest Total Plate Count throughout the storage period.

According to [19] paper packaging can increase the growth of bacteria and fungi due to paper's ability to absorb water. These results are consistent with the findings reported by [11]. In their study, cookies packaged in different types of packaging showed varying bacterial counts, with paper packaging having a TPC value of 5.44 log CFU/g, PE plastic 4.55 log CFU/g, and PP plastic 4.27 log CFU/g. Packaging provides environmental conditions for the packaged material, and these conditions are determined by the water vapor pressure of the food at storage temperature and the durability of the packaging.

From the data obtained, the Total Plate Count (TPC) in each treatment met the requirements SNI 8217:2015 Dry noodles. The TPC requirement for yellow pumpkin noodles is a maximum of 106. Lower microbial growth in aluminum foil packaging was associated with reduced oxygen and moisture penetration, creating less favorable conditions for bacterial proliferation.

3.3. *Escherichia coli*

Based on the *Escherichia coli* test on day 0, all treatments showed no *Escherichia coli* bacteria in the yellow pumpkin noodles during packaging; on the 9th day of testing, no *Escherichia coli* bacteria were detected in the yellow pumpkin noodles during packaging. Table 4 presents the average *Escherichia coli* test results for yellow pumpkin noodles.

Table 4. Average number of *Escherichia coli* bacteria in pumpkin noodles

Packaging types	<i>Escherichia coli</i> (MPN/g)	<i>Escherichia coli</i> (MPN/g)
	Day-0	Day-9
A (PP)	< 3	< 3
B (PE)	< 3	< 3
C (PS)	< 3	< 3
D (Aluminium Foil)	< 3	< 3
E (Paper)	< 3	< 3

The research results revealed inhibition of *Escherichia coli* bacterial growth in pumpkin noodles in various packaging. This was evident in the study's results, which showed no color change in the packaged product (pumpkin noodles). Pathogenic bacteria include *Escherichia coli*, *Salmonella* sp., and *Staphylococcus aureus*, among others [20]. The results of this study showed that pumpkin noodles stored in various types of packaging were free from *Escherichia coli* contamination. This may be attributed to the hygienic conditions of the equipment, storage area, knives, and handlers during processing.

According to [22] which causes samples to be contaminated with bacteria caused by knives, spoons used to cut raw materials such as raw meat that are contaminated by pathogenic bacteria. If the equipment is used without being washed first to cut cooked food, it will be contaminated by the equipment, by transferring pathogens which can cause risks to those who consume the food.

The total number of *Escherichia coli* bacteria in the pumpkin noodles produced on the 9th day still meets the quality requirements for pumpkin noodles set by the Indonesian National Standard.SNI 8217:2015 that is, a maximum of 10 APM/g.

3.4. *Mold Count*

The results of the diversity analysis showed that different types of pumpkin noodle packaging had a highly significant effect ($P < 0.01$) on mold counts. The average mold counts for pumpkin noodles in various packaging are presented in Table 5.

Table 5. Average mold count various packages on pumpkin noodles

Packaging types	Mold count (CFU/g)	Mold count (CFU/g)
	Day-0	Day-9
A (PP)	1.5×10^3	4.7×10^3 b
B (PE)	1.2×10^3	7.0×10^3 b
C (PS)	1.1×10^3	8.8×10^3 c
D (Aluminium Foil)	1.3×10^3	2.5×10^3 a
E (Paper)	1.1×10^3	9.6×10^3 c

Note: Mean values followed by different letters indicate significant differences ($P < 0.01$)

Table 4 shows the number of molds on pumpkin noodles. The number of mold additions in each treatment differs in each package. The highest number of molds was found in pumpkin noodles with paper packaging, namely 9.6×10^3 on the 9th day, while the lowest number of molds was found in pumpkin noodles with aluminum foil packaging, with a value of 2.5×10^3

From the results above, it can be seen that aluminum foil packaging has the ability to withstand/inhibit mold growth longer than other packaging. According to [14], aluminum foil is a type

of packaging that has the characteristics of being odorless, tasteless, harmless and hygienic, not easily causing the growth of bacteria and fungi, while paper packaging is a type of packaging that has a higher increase in microbes because paper is a type of packaging that easily absorbs water so that it can accelerate the growth of microbes so that the packaged food is not durable.

According to [23], paper packaging is sensitive to water and easily affected by environmental humidity, which can accelerate mould growth. This is consistent with research [6]; the number of moulds on papaya dodol continued to increase during packaging. Aluminum foil packaging inhibited mold growth by 180 colonies/g, while paper packaging only inhibited mold growth by 530 colonies/g.

The highest mold count was found in yellow pumpkin noodles stored in paper packaging, namely 9.6×10^3 , while the lowest mold count was found in yellow pumpkin noodles in various aluminum foil packaging, namely 2.5×10^3 .

Mold growth is strongly associated with an increase in water activity (aw), which reflects the availability of free water required for fungal metabolism and reproduction. During storage, moisture uptake by the product increased the water activity of the noodles, particularly in paper packaging with poor moisture barrier properties. The higher water activity created favorable conditions for mold spore germination and fungal proliferation. Conversely, aluminum foil effectively minimized moisture transfer from the surrounding environment, thereby maintaining lower water activity and suppressing mold growth throughout the storage period.

The effectiveness of packaging materials in controlling mold development depends largely on their barrier properties against water vapor and oxygen transmission. Packaging materials with low water vapor transmission rates (WVTR), such as aluminum foil, reduce moisture adsorption and maintain product stability during storage. In contrast, paper packaging allows greater moisture diffusion, accelerating fungal growth and reducing product shelf life. The mold count obtained in this study met the requirements for yellow pumpkin noodles SNI 8217-2015.

These results are consistent with the moisture content and Total Plate Count analyses, indicating that packaging materials with superior moisture and oxygen barrier properties were more effective in maintaining the microbiological stability of fortified pumpkin noodles during storage.

3.5. *Salmonella* sp

Salmonella sp. in pumpkin noodle samples packaged in various packaging types was identified through several stages, including pre-enrichment, enrichment, selective media isolation, and biochemical testing. The results of the identification are presented in Table 6

Table 6. *Salmonella* sp test results pumpkin noodles

Packaging types	<i>Salmonella</i> sp. Day-0	<i>Salmonella</i> sp. Day-9
A (PP)	Negative/25g	Negative/25g
B (PE)	Negative/25g	Negative/25g
C (PS)	Negative/25g	Negative/25g
D (Aluminium Foil)	Negative/25g	Negative/25g
E (Paper)	Negative/25g	Negative/25g

Based on Table 5, the identification of *Salmonella* sp. in yellow pumpkin noodles packaged in five different types of

packaging, namely A (PP packaging), B (PE packaging), C (PS packaging), D (aluminum foil packaging), and E (paper packaging), showed negative results for all samples after testing. This indicates that the pumpkin noodles stored in various packaging types were free from *Salmonella* sp. contamination.

According to [18], pathogenic bacterial contamination may occur when knives or other equipment previously used to cut raw materials, such as raw meat contaminated with pathogenic bacteria, are reused without proper cleaning. If the equipment is reused without proper washing to handle processed or ready-to-eat food, cross-contamination may occur through the transfer of pathogenic microorganisms, which can pose health risks to consumers.

Foods contaminated with small amounts of *Salmonella* sp. generally do not show changes in appearance, taste, or odor. However, if the number of bacteria is high, changes in the appearance, taste, and characteristic odor of the food may occur. According to SNI 7388:2009 concerning the maximum limit of microbial contamination in food, *Salmonella* sp. must be negative in 25 g of food samples.

The results of this study indicate that yellow pumpkin noodles stored in various packaging types, namely A (PP packaging), B (PE packaging), C (PS packaging), D (aluminum foil packaging), and E (paper packaging), were free from *Salmonella* sp. growth during storage up to the 9th day.

4. CONCLUSION

The results of this study showed that the storage of yellow pumpkin noodles packaged in polypropylene (PP), polyethylene (PE), polystyrene (PS), aluminum foil, and paper significantly affected moisture content, Total Plate Count (TPC), and mold count ($P < 0.01$). Among the packaging materials tested, aluminum foil packaging (treatment D) demonstrated the best ability to maintain product quality, resulting in a moisture content of 9.64%, Total Plate Count (TPC) of 6.9×10^3 CFU/g, *E. coli* < 3 MPN/g, mold count of 2.5×10^3 CFU/g, and *Salmonella* sp. negative/25 g after 9 days of storage. All values complied with the requirements of SNI 8217:2015 for dry noodles and SNI 7388:2009 for microbiological safety. Aluminum foil packaging demonstrated superior barrier properties and was the most effective packaging material for maintaining microbiological quality of tuna bone flour-fortified pumpkin noodles during nine days of storage. Although shelf-life estimation suggested a longer storage period, further validation through extended storage experiments involving physicochemical, microbiological, and sensory analyses is necessary before confirming the predicted shelf life. Based on the predictive model used in this study, aluminum foil packaging was estimated to provide the longest shelf life. However, this prediction requires validation through long-term storage experiments. This study was limited to a 9-day observation period under room-temperature storage conditions. Therefore, further studies involving longer storage periods, physicochemical quality evaluation, sensory analysis, and validation of the predicted shelf life are recommended to provide a more comprehensive assessment of product stability and storage performance.

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