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Determination of β-Carotene in Carrot-Fortified Snakehead Fish (Channa striata) Pempek after Sequential Freezing–Thermal Processing and Vacuum Packaging by HPLC

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This study is the first to quantitatively determine the final retained β-carotene content in carrot-fortified snakehead fish pempek after integrated sequential freezing–thermal processing and vacuum packaging using validated HPLC-PDA analysis under conditions representative of small-scale traditional production.

A B S T R A C T

This study evaluated the effect of carrot puree concentration on the final β-carotene content of snakehead fish (Channa striata) pempek subjected to integrated freezing–thermal–vacuum processing. Four formulations were prepared with varying carrot concentrations (0 g, 150 g, 300 g, and 500 g). β-Carotene was quantified using a validated HPLC-PDA method with a C18 column and detection at 450 nm. The calibration curve showed excellent linearity ($R^2 = 0.9986$), with calculated limits of detection (LOD) and quantification (LOQ) of 0.13 mg/L and 0.40 mg/L, respectively. The measured β-carotene concentrations ranged from 0.27 to 0.67 mg/kg, with the highest value observed in the 500 g carrot formulation. Statistical analysis confirmed a significant effect of carrot concentration on β-carotene content ($p < 0.05$), demonstrating a clear dose-dependent relationship. These findings support the application of carrot puree as a functional fortification ingredient in traditional fish-based products. Future studies may investigate optimized thermal conditions or protective strategies to further improve carotenoid stability during processing.

Contribution to Sustainable Development Goals (SDGs):
SDG 2: Zero Hunger
SDG 3: Good Health and Well-being

1. INTRODUCTION

1.1. Research Background

Pempek is a traditional fish-based food product widely consumed in Indonesia and has considerable potential to be developed into a nutritionally enhanced functional food. Snakehead fish (*Channa striata*) is recognized as a high-quality source of animal protein, containing essential amino acids that support tissue growth and metabolic functions. However, despite their high protein content, fish-based products generally contribute limited amounts of micronutrients and bioactive compounds, thereby restricting their role in addressing micronutrient deficiencies [1].

Carrots (*Daucus carota* L.) are a natural source of β-carotene, dietary fiber, and essential minerals. β-Carotene functions as a major dietary precursor of vitamin A, which plays a critical role in vision, immune regulation, and epithelial integrity. Vitamin A deficiency (VAD) remains a public health concern in several developing regions, including Indonesia. In 2022, the prevalence of VAD among children under five in Indonesia reached 19.5%,

indicating that nearly one in five toddlers was affected. Clinically, VAD may manifest as night blindness and xerophthalmia, and in severe cases may progress to irreversible ocular damage. Beyond ocular complications, VAD compromises immune function and increases susceptibility to infectious diseases. Despite national supplementation and fortification programs, the burden of VAD remains substantial [2,3].

Food-based strategies incorporating provitamin A–rich ingredients into commonly consumed products represent a sustainable intervention approach [4]. Within this context, fortifying fish-based products such as pempek with carrots offers potential to enhance nutritional value while improving functional and sensory characteristics. In addition to increasing β-carotene content, carrot incorporation may improve product colour and reduce fishy odour, thereby supporting consumer acceptability [5,6].

Nevertheless, β-carotene is highly susceptible to degradation during processing and storage. Thermal exposure, oxygen, and light accelerate carotenoid degradation through oxidative reactions and heat-induced isomerisation. Previous kinetic studies have demonstrated that β-carotene degradation under elevated temperatures follows first-order reaction kinetics, with significant losses attributed to oxidative and structural transformation pathways [7]. These findings underscore the inherent instability of β-carotene under conditions commonly applied in fish-based food processing.

Various preservation technologies, including freezing, thermal treatment, and vacuum packaging, have been employed to improve nutrient stability. Freezing reduces chemical and enzymatic reaction rates by limiting molecular mobility, although ice crystal formation may alter cellular structure and influence nutrient retention during subsequent heating [8,9]. Thermal processing is essential to ensure microbiological safety and desirable texture in fish-based products, but may accelerate the degradation of heat-sensitive compounds, such as β-carotene. Vacuum packaging reduces oxygen availability by lowering oxygen partial pressure, thereby minimizing oxidative degradation and potentially enhancing carotenoid stability [10].

However, quantitative data reporting the final β-carotene concentration in composite fish–vegetable traditional products after sequential freezing–thermal processing followed by vacuum packaging remain limited. Most previous studies have primarily focused on carotenoid degradation kinetics in plant-based systems or single-stage thermal treatments, with limited attention to multi-stage integrated processing applied in traditional composite matrices.

To the best of our knowledge, only a few studies have quantitatively determined the final β-carotene concentration in carrot-fortified snakehead fish (Channa striata) pempek processed under integrated freezing–thermal–vacuum conditions, using validated HPLC-PDA analysis representative of small-scale traditional production. Therefore, this research aims to evaluate the effect of carrot concentration on the final β-carotene content in carrot-fortified snakehead fish pempek after sequential processing.

1.2. Literature Review

1.2.1. Carotenoids and Their Nutritional Significance

Carotenoids are a large group of naturally occurring lipophilic pigments widely distributed in plants, algae, and photosynthetic microorganisms. More than 600 carotenoids have been identified, although only a limited number are commonly present in the human diet. Structurally, carotenoids are tetraterpenoid compounds composed of 40 carbon atoms with an extensive system of conjugated double bonds. This conjugated structure is responsible for their characteristic yellow-to-red colouration and antioxidant properties [4].

Carotenoids are classified into two main groups: carotenes, which consist solely of hydrocarbons, and xanthophylls, which contain oxygenated functional groups (Figure 1). Among the carotenes, β-carotene is the most nutritionally significant compound due to its provitamin A activity. Unlike other carotenoids such as lutein and lycopene, β-carotene can be enzymatically converted into vitamin A in the human body [4].

In food systems, carotenoids are naturally embedded within plant cellular structures. When incorporated into composite matrices, such as fish-based products, their behaviour may be influenced by interactions with lipids and proteins, thereby affecting extractability, stability, and measurable concentrations after processing. Therefore, understanding the characteristics of β-carotene in composite food matrices is essential when evaluating its nutritional contribution [4].

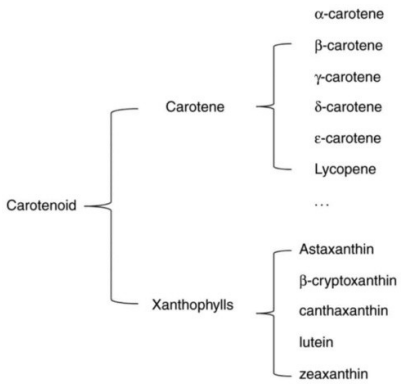

Fig 1. Classification of carotenoids.

1.2.2. Beta-Carotene as a Provitamin A and Antioxidant

β-Carotene functions as a major dietary precursor of vitamin A (retinol). After ingestion, it is absorbed in the small intestine and cleaved by β-carotene-15,15′-monooxygenase (CMOI) to produce retinal, which is subsequently reduced to retinol or oxidized to retinoic acid (Figure 2). Vitamin A plays a fundamental role in visual function, immune response, epithelial integrity, and cellular differentiation. Insufficient intake of provitamin A carotenoids contributes to vitamin A deficiency (VAD), which remains a significant public health concern in developing countries, including Indonesia [4].

In addition to its role as a vitamin A precursor, β-carotene exhibits antioxidant activity due to its conjugated polyene chain, which enables it to quench singlet oxygen and neutralize free radicals (Figure 2). This antioxidant property is particularly relevant in food systems containing lipids, where oxidative reactions may lead to nutrient degradation and quality deterioration [4].

However, the same structural characteristics that confer antioxidant capacity also render β-carotene susceptible to degradation when exposed to heat, oxygen, and light. Oxidative cleavage and trans–cis isomerization may reduce its measurable concentration and potentially affect its provitamin A activity [4].

It is important to distinguish between β-carotene concentration and bioavailability. While processing conditions may decrease total β-carotene levels, structural disruption of plant tissues can enhance carotenoid release from the matrix, potentially improving extractability and bioaccessibility. Therefore, measured concentration after processing does not always directly correspond to physiological functionality, and both degradation and matrix-related effects should be considered when interpreting processing outcomes [4].

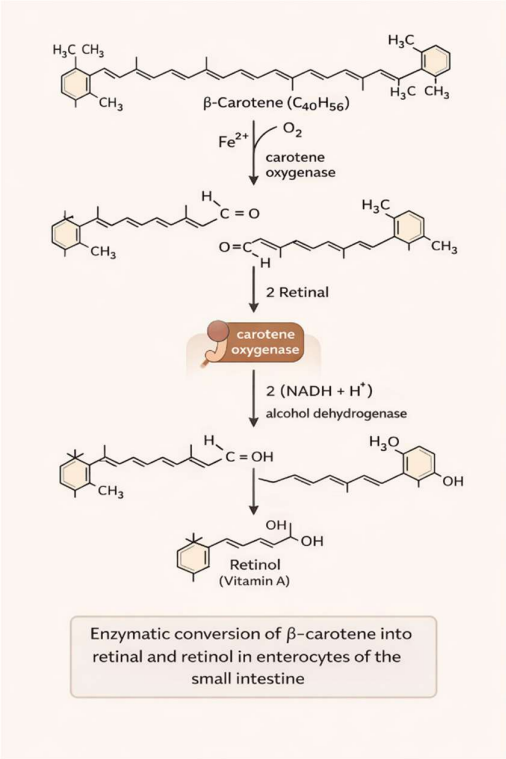

Fig 2. Enzymatic conversion of β-carotene to retinol

1.2.3. Stability of Beta-Carotene During Food Processing

Carotenoids are sensitive to environmental conditions such as heat, oxygen, and light due to their highly unsaturated polyene structure. Processing treatments may induce oxidative reactions and trans–cis isomerization, potentially reducing measurable β-carotene concentration and provitamin A activity [3]. Thermal exposure, particularly at elevated temperatures, accelerates the oxidative cleavage of the polyene chain, leading to pigment degradation.

Freezing treatment may reduce enzymatic and chemical reaction rates by limiting molecular mobility; however, ice crystal formation can disrupt cellular structures and modify matrix integrity [3]. Such structural changes may influence carotenoid distribution and subsequent behaviour during further heat exposure.

Oxygen availability plays a critical role in carotenoid stability. Oxidative reactions occur more rapidly in the presence of oxygen, leading to decreased carotenoid levels. Packaging systems such as vacuum packaging reduce the partial pressure of oxygen and may help limit post-processing oxidative losses in heat-treated products [3].

In composite food matrices, interactions between lipids, proteins, and carotenoids further influence nutrient behavior. The presence of lipids may enhance carotenoid solubilization and extractability, while matrix disruption during processing may simultaneously increase exposure to oxygen and heat. Therefore, evaluating the final measurable β-carotene concentration after integrated processing becomes essential to determine the actual nutritional contribution of fortified traditional food products [3].

2. RESEARCH OBJECTIVE

This study aims to evaluate the effect of carrot concentration on the final β-carotene content of carrot-fortified snakehead fish (Channa striata) pempek after sequential freezing–thermal processing followed by vacuum packaging. The study quantitatively assesses β-carotene retention using validated HPLC-PDA analysis under integrated processing conditions representative of small-scale traditional production.

3. MATERIALS AND METHODS

3.1. Materials

Fresh snakehead fish (*Channa striata*), tapioca flour, monosodium glutamate (MSG), fresh carrots (*Daucus carota*), and salt were purchased from a traditional market in Palembang, South Sumatra, Indonesia.

Analytical-grade chemicals used for β-carotene analysis included β-carotene standard (Sigma-Aldrich), petroleum benzene, methyl tert-butyl ether (MTBE), ethanol, methanol, n-hexane, anhydrous sodium sulfate (Na_2SO_4), and distilled water.

3.2. Formulation of Carrot Pempek

The formulation of carrot-enriched pempek is presented in Table 1. Pempek dough was prepared by mixing minced snakehead fish, tapioca flour, water, and mashed carrot according to treatment levels:

- P1 = 150 g carrot
- P2 = 300 g carrot
- P3 = 500 g carrot

Salt and MSG were added uniformly to the mixture. The dough was shaped into cylindrical forms and boiled in water until fully cooked.

Table 1. Ingredient composition in carrot pempek production

Ingredient	P1 (g)	P2 (g)	P3 (g)
Snakehead fish	1000	1000	1000
Tapioca flour	800	800	800
Water (mL)	500	500	500
Carrot	150	300	500
MSG	20	20	20
Salt	40	40	40

A control sample (P0) was prepared without carrot addition.

3.3. Sequential Freezing–Thermal Treatment and Vacuum Packaging

After boiling, the pempek samples were subjected to sequential freezing–thermal treatment. Cooked samples were stored in a conventional freezer (−18°C) for 48 hours. Subsequently, frozen samples were transferred to a hot-air oven and heated at 100°C for 1 hour (primary thermal treatment), followed by secondary heating at 50°C for 15 minutes. After thermal treatment, the samples were vacuum-packaged using a commercial vacuum-

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sealing machine to minimise oxygen exposure. β-Carotene analysis was conducted one day after packaging.

3.4. Extraction of β-Carotene

Five grams of finely chopped pempek sample were placed into an Erlenmeyer flask. Fifteen milliliters of n-hexane:ethanol (2:1 v/v) solvent mixture and 1 g of anhydrous Na_2SO_4 were added. The mixture was vortexed for 10 minutes and centrifuged at 4000 rpm for 10 minutes. The upper n-hexane layer containing β-carotene was collected and filtered through Whatman filter paper or a 0.45 µm membrane filter prior to HPLC analysis [11].

3.5. Preparation of β-Carotene Standard Solution

Approximately 0.05 g of β-carotene standard was accurately weighed and dissolved in petroleum benzene in a 50 mL volumetric flask to obtain a 1000 ppm stock solution. One milliliter of the stock solution was diluted to 10 mL with solvent to prepare a 100 ppm working standard. Calibration curves were constructed prior to sample analysis.

3.6. HPLC Analysis

β-Carotene quantification was performed using an HPLC system (Agilent 1120 series) equipped with a C18 column and photodiode array (PDA) detector. The mobile phase was delivered at a flow rate of 1 mL/min. Detection wavelength was determined using a UV-Vis spectrophotometer prior to analysis.

Samples and standards were injected at a volume of 20 µL. Identification of β-carotene was based on comparison of the retention time with that of the standard solution. Method precision was evaluated by five repeated injections of the standard solution and expressed as relative standard deviation (RSD) [11,12].

3.7. Statistical Analysis

All treatments were performed in triplicate. Data were analysed using one-way analysis of variance (ANOVA) at the 5% significance level ($p < 0.05$). Tukey's test was applied for post hoc comparisons when significant differences were observed. Pearson correlation analysis was conducted to determine relationships between variables.

4. RESULT AND DISCUSSION

4.1. Physical Characteristics of Carrot Pempek

The incorporation of carrot puree significantly affected the physical characteristics of pempek, including colour, aroma, flavour, and texture (Table 2). The observed colour changes were primarily attributed to β-carotene, the major carotenoid pigment in carrots, which imparts a bright orange hue.

Colour intensity increased proportionally with carrot concentration. The P3 sample (500 g carrot) exhibited the deepest orange coloration, while P1 showed a lighter orange tone. The control sample (P0) retained a greyish-white appearance typical of conventional fish-based pempek.

Aroma evaluation revealed a gradual reduction in fishy odour with increasing carrot concentration. This may be attributed to the natural sweetness and volatile compounds present in carrot, which masked fish-derived odour compounds. In terms of flavour, increasing carrot levels contributed to a sweeter taste profile, particularly in P3. Texture remained chewy in P1 and P2 but became slightly softer in P3, likely due to increased moisture and fibre content from carrot puree.

These findings indicate that carrot fortification enhances not only nutritional value but also sensory attributes, potentially improving consumer acceptability [6].

Table 2. Characteristics of Carrot Pempek

Characteristics	P0	P1	P2	P3
Colour	Greyish White	Cantaloupe	Orange	Cider
Aroma	Strong Fishy	Less Fishy	No Fishy Aroma	Slight Carrot Aroma
Flavour	Quite Savory	Savory	Savory and Sweet	Sweet
Texture	Chewy	Chewy	Chewy	Soft

4.2. Sequential Freezing–Thermal Treatment (Current Method)

Following boiling, samples underwent sequential freezing–thermal treatment. Freezing at −18°C for 48 hours preserved product structure and inhibited microbial growth. During freezing, water crystallization may have altered the microstructure, potentially influencing moisture distribution and subsequent drying behaviour.

Primary heating at 100°C for 1 hour effectively removed surface moisture. However, exposure to elevated temperature likely initiated partial thermal degradation of β-carotene. Carotenoids are susceptible to oxidative cleavage and trans–cis isomerisation under heat and oxygen, which may reduce their provitamin A activity [13].

The secondary heating stage at 50°C for 15 minutes served as a mild finishing treatment to reduce residual moisture without causing excessive structural damage. At this lower temperature, β-carotene degradation is less pronounced compared to high-temperature exposure [14].

Vacuum packaging applied after thermal treatment played a critical role in limiting oxygen availability, thereby reducing post-processing oxidative degradation. Reduction in water activity and oxygen exposure contributes to extended shelf stability and improved nutrient retention [15].

4.3. HPLC Method Validation Using Standard Solution

β-Carotene quantification was performed using HPLC-PDA with a C18 column (250 mm × 4.6 mm; 5 µm particle size). The selection of a reverse-phase C18 column was appropriate due to the non-polar and lipophilic nature of β-carotene [16].

The mobile phase consisting of methanol:MTBE (80:20, v/v) provided optimal peak symmetry and stable retention time (approximately 9 minutes). Detection was carried out at 450 nm, corresponding to the maximum absorption wavelength of β-carotene [17].

The calibration curve constructed from standard concentrations showed excellent linearity, with a coefficient of determination (R^2) of 0.9986. The regression equation showed strong correlation between concentration and peak area, indicating high analytical reliability and suitability for quantitative determination (Figure 3). Repeated standard injections produced minimal retention time variation (±0.1 min), confirming system stability and method precision.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the regression response (σ) and the slope (S) of the calibration curve according to ICH guidelines, using the equations LOD = 3.3σ/S and LOQ = 10σ/S. The calculated LOD and LOQ values were 0.13 mg/L and 0.40 mg/L, respectively. These results indicate adequate sensitivity of the method for detecting and quantifying β-carotene within the concentration range observed in the fortified samples.

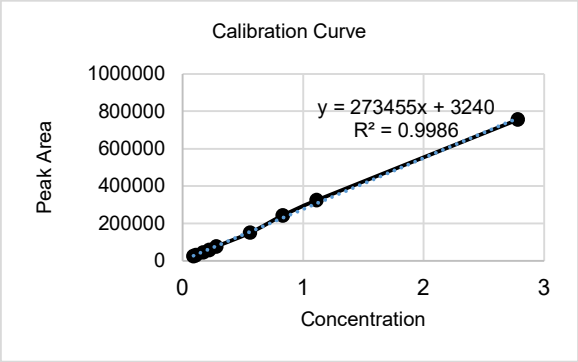

Fig 3. The Curve of standard solution (β-Carotene).

4.4. Effect of Sequential Freezing–Thermal Treatment on β-Carotene Content Using HPLC

The measured β-carotene concentrations in processed carrot pempek ranged from 0.27 to 0.67 mg/kg, with the highest value observed in sample P3 (500 g carrot formulation). The increasing trend confirms that carrot concentration directly influenced the final β-carotene content in the composite fish-based matrix.

Although β-carotene was successfully detected in all fortified samples, the measured concentrations were relatively low. This may be attributed to carotenoids' susceptibility to thermal degradation and oxidative reactions during exposure at 100°C. Heat-induced trans–cis isomerisation and oxidative cleavage of the polyene chain are known mechanisms of carotenoid instability.

Freezing at −18°C before thermal treatment may have reduced enzymatic activity during storage; however, ice crystal formation can disrupt cellular structures within the carrot–fish matrix. Such structural changes may increase carotenoid exposure to oxygen during subsequent heating, thereby limiting the protective effect of freezing against thermal degradation.

Although freezing at −18 °C can slow enzymatic and oxidative reactions during storage, it does not chemically stabilize β-carotene against subsequent thermal exposure. Freezing primarily affects reaction kinetics at low temperatures, and the formation of ice crystals can disrupt cellular structures, potentially increasing the exposure of carotenoid molecules during later heating stages. Research indicates that thermal processing at elevated temperatures (e.g., 90–150 °C) promotes isomerization and oxidative degradation of β-carotene, even when preceded by freezing. These changes are driven by temperature-dependent reactions that are not prevented by prior freezing, highlighting that heat exposure is the dominant factor influencing carotenoid stability in food matrices [18].

Vacuum packaging applied after thermal treatment likely minimized post-processing oxidative losses by reducing oxygen availability. However, degradation occurring during the high-temperature stage could not be reversed. Therefore, while sequential freezing–thermal processing combined with vacuum packaging resulted in measurable β-carotene levels, optimization of thermal intensity remains essential to improve carotenoid stability.

4.5. Statistical Analysis

One-way ANOVA revealed highly significant differences among treatments ($F = 311.70$; $p < 0.001$). The extremely low p-value (8.66×10^{-7}) confirms that carrot concentration significantly affected β-carotene content. Tukey's HSD post-hoc test demonstrated significant differences between all treatment pairs (P1 vs P2, P1 vs P3, and P2 vs P3; $p < 0.05$). This indicates a clear dose-dependent relationship between carrot concentration and β-carotene content.

4.6. Comparison Between Theoretical and Experimental β-Carotene Content

The literature reports that fresh carrots contain considerable amounts of β-carotene, typically ranging from approximately 2.3 to 9.5 mg per 100 g fresh weight, depending on cultivar and growing conditions [19]. In the present study, β-carotene was quantified in a processed composite matrix containing fish, starch, water, and carrot puree. Because literature values represent β-carotene content in fresh carrot tissue alone, whereas this study measured β-carotene within a multi-component processed product, direct numerical comparison is not strictly equivalent. The measured concentrations therefore reflect the combined influence of formulation proportion and integrated processing conditions rather than representing the intrinsic β-carotene level of carrot tissue. Consequently, literature values are provided solely as contextual reference and not as a basis for quantitative evaluation of carotenoid loss or degradation.

5. CONCLUSION

This study demonstrated that carrot puree fortification significantly increased the measurable β-carotene concentration in snakehead fish (Channa striata) pempek after integrated freezing–thermal–vacuum processing. β-Carotene was successfully quantified using a validated HPLC-PDA method with good linearity and analytical sensitivity. Increasing carrot concentration resulted in a dose-dependent increase in final β-carotene content, as confirmed by statistical analysis ($p < 0.05$). Detectable levels of β-carotene were maintained in all fortified treatments, indicating the feasibility of incorporating carrot puree into traditional fish-based products. Future studies should explore lower thermal intensity, inert-atmosphere heating, or encapsulation strategies to enhance carotenoid stability and optimize nutrient retention in processed composite foods.

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