

Propolis as a Natural Preservative for Frozen Fish Burgers: A Kinetic Study of Lipid Oxidation and Microbial Growth

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ABSTRACT

The kinetic mechanisms governing fatty acid (FA) degradation and microbial growth rate in seafood products are of paramount importance. This study evaluated the interactive effects of propolis incorporation at varying concentrations and storage duration on fatty acid oxidation kinetics and its inhibitory role in suppressing bacterial and fungal proliferation in frozen fish burger patties (FFB) stored at -18°C . The rates of fatty acid degradation and microbial growth in FFB during the storage period were found to follow zero-order kinetic models. After three months of storage, the treatment group containing 0.4% propolis (P-IV) exhibited the lowest growth rates (\log_{10} CFU/g) for total viable count (3.66) and fungi (2.43). Correspondingly, this group displayed the lowest rate of peroxide value increase ($k_0 = 0.0462$ meq O_2/kg oil/day), indicative of minimal fatty acid oxidation, and received the highest sensory evaluation scores. The results demonstrate that incorporating 0.4% propolis into FFB and storing them at -18°C can effectively retard lipid oxidation and microbial proliferation, while concomitantly enhancing sensory quality for up to 86 days, which can be considered the optimal shelf life under these conditions.

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1. Introduction

Processed meat products, such as burger patties, are highly perishable and susceptible to quality deterioration due to their preparation processes, including cutting, grinding, mixing, and forming. These processes expose a larger surface area of the meat to oxygen and microorganisms, accelerating oxidation and microbial growth while causing mechanical injury to tissues. Consequently, these factors promote biochemical changes and microbial contamination, deteriorating quality (1). Assessing and predicting fish burger freshness (FBF) is crucial for ensuring safety, quality, and shelf life, as well as for reducing food waste and optimizing production and distribution processes (2). FBF can be measured using various indicators, including sensory attributes, pH, water activity (a_w), total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), and microbial counts (3). However, measuring these indicators is often time-consuming

and requires skilled personnel and expensive equipment. Thus, there is a need for rapid, non-destructive, and reliable methods to monitor and predict FBF (4). One emerging approach for FBF prediction is based on mathematical modeling, which employs mathematical equations to describe changes in quality indicators as a function of time and other influencing factors, such as temperature, oxygen, packaging, and additives (5). Various models can estimate the optimal storage time and conditions of burger patties based on their quality and safety parameters. Among these, the most well-known are kinetic models, including the Arrhenius, Q_{10} , and Weibull models. For instance, Quevedo et al. (6) investigated quality changes in frozen industrial burgers stored at different temperatures, using the Weibull model to fit the kinetics of oxidative rancidity, color, texture, and other indicators. Their results confirmed that the shelf life of burgers was primarily affected by storage temperature and oxidation processes. Another modeling technique successfully utilized to predict the shelf

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life of chilled/supercooled pork is the entropy weight method (EWM). This method employs a holistic quality indicator that combines various quality indexes (e.g., microbial, chemical, and sensory) using a weighting method (7). Regression techniques such as partial least squares (PLS) can also estimate shelf life. PLSR can handle multiple predictors and responses, address collinearity and noise, and extract latent variables that capture the relationship between quality attributes and storage conditions (8). Marques et al. (9) used a PLS model to predict the sensory rancid taste, pH, and TBARS of grass carp burgers from the RGB pattern of digital images. They reported high coefficients of determination (R^2 -value) and low root mean square errors (RMSE) of prediction, indicating good agreement between predicted and observed values. Furthermore, Cui et al. (10) reviewed the performance of various shelf-life prediction models in the food industry, such as neural network models and kinetic models, conducting a horizontal comparison of modeling approaches. These studies demonstrate that mathematical modeling for freshness assessment can be applied at different levels of complexity and detail, ranging from single quality indicators to multiple quality attributes, from raw to processed products in fish and seafood, and from static to dynamic conditions. Nevertheless, kinetic models are recognized as a superior technique for predicting and estimating the shelf life of food products, such as fish burgers, because they can capture the effects of environmental factors, provide mechanistic insights, and facilitate prediction under different storage conditions (11). This study aimed to elucidate the quality attributes of fish burgers during storage by applying kinetic modeling. The factors influencing quality were the concentrations of propolis, a powerful antioxidant, and the duration of storage.

2.2. Materials and methods

2.1. Raw material preparation

Fresh rainbow trout (*Oncorhynchus mykiss*) with mean weight (800 ± 100 g) and length (30 ± 2 cm) were purchased from a local market and transported under cold-chain conditions ($1-4^\circ\text{C}$) to the Azad University laboratory. Raw propolis (*Apis mellifera* L.) was obtained from a Bee-Breeding Center.

2.2. Production of fish burger patties

The fish were initially washed with tap water. After removing the heads, they were peeled, deboned, and eviscerated. The lean meat was cut into fillets and minced using a homemade meat grinder (Panasonic, MK-ZJ3500, Japan) to achieve 5 mm particle sizes. Ingredients, including salt (2%), mixed spices (2%, comprising curry powder, pepper, and turmeric), bread powder (5%), sugar (0.5%), garlic powder (1.5%), and onion powder (4.0%), were uniformly mixed as supplements. These ingredients were subjected to ultraviolet light (200-280 nm) for 20 minutes for decontamination. Following the standard fish burger recipe, 85% (w/w) of minced fish fillet was combined with 15% (w/w)

of additives to form a consistent paste. The paste was then mixed with propolis powder at various concentrations (0 to 0.4%). Circular burgers (1 cm thickness, 10 cm diameter, and $100 \text{ g} \pm 5$ weight) were formed from the fish pastes. The burgers were manually packed in polyethylene bags and frozen at -18°C for three months. The quality assessment of frozen fish burgers (FFB) was conducted at 0, 30, 60, and 90 days (3).

2.3. Measuring peroxide values of FFB's oil during storage

Oil was extracted from each FFB sample using a Soxhlet extractor at temperatures below 20°C (to inhibit lipid oxidation), following the method described by Shabani et al. (3). The peroxide value (PV) of the extracted oil was determined using the iodometric titration method.

2.4. Microbiological analysis

Microbiological analyses were performed on samples at 0, 30, 60, and 90 days during storage at -18°C . The total viable count (TVC) of each sample was enumerated using plate count agar (Fater-riz-pardaz, B630, Iran), followed by incubation at 37°C for 48 h (12). Fungi (mold and yeast) in FFB samples were enumerated using Yeast Extract Glucose Chloramphenicol Agar after inoculation using the surface plate technique. The inoculated plates were incubated at 25°C for 5-7 days before enumeration (13). After the incubation period, plates with 30 to 300 colonies were counted. Results were expressed as Log_{10} CFU/g of the sample.

2.5. Data fitting and model validation

2.5.1. quality kinetic modeling of FFB in frozen storage

The radical oxidation mechanism (formation of hydroperoxides) and microbial growth rate (MGR) in FFB are the most destructive reactions leading to product quality decline during storage. Changes in PV and TVC were monitored during the storage period to determine microbial and oxidation reaction orders, as well as to estimate the shelf life of FFB. Table 1 shows different models used to display changes in increasing PVs (or TVCs) of different FFB samples during storage and to determine their reaction orders (from zero-order to second-order). The coefficient of determination (R^2) and mean relative deviation (P%) were used to identify the best model for the order of lipid oxidation reaction (or MGR). The R^2 -value should be higher for a quality fit, and the P% should be lower. These parameters were calculated using Equations (1) and (2). Data fitting was performed using Microsoft Excel 2019.

$$R^2 = 1 - \frac{\sum_{i=1}^N (\theta_{p,i} - \theta_{e,i})^2}{\sum_{i=1}^N (\theta_{p,i} + \theta_{e,i})^2} \quad (1)$$

$$P(\%) = \frac{100}{N} \sum_{i=1}^N |\theta_{p,i} - \theta_{e,i}| \quad (2)$$

Where, $\theta_{p,i}$ is predicted data, $\theta_{e,i}$ is observed data, $\bar{\theta}_{p,i}$ is the average of predicted data, and N is the number of observations (14, 15).

2.5.2. Shelf-life prediction of FFB in frozen storage

Processing and storage conditions affect the shelf life of produced fish burgers. Generally, after determining the key parameters, the shelf life of FFB can be calculated using the equations presented in Table 1 (11).

2.6. Consumer preference

The sensory quality of FFB (after three months of storage) was assessed by 15 trained and experienced panelists (men and women) aged 20 to 30 years. They were given random samples of raw fish burgers (weighing about 20 g) labeled with 3-digit random numbers. Panelists rated sensory attributes (mainly

aroma), organoleptic characteristics (general appearance, texture, and visual color), and overall acceptability of FFB according to the method described by Stone and Sidel (16). A 5-point hedonic scale (1 = extremely dislike, 2 = dislike, 3 = neutral, 4 = like, and 5 = extremely like) was used to score different properties, and their means were considered for final evaluation (17, 18).

2.7. Data analysis

Analysis of variance (ANOVA) and least significant difference (LSD) tests for different treatments were performed using Statistix version 8 (Analytical Software Inc., Tallahassee, FL 32312, USA) at a 99% confidence level.

Table 1. The different forms of quality kinetics models and their shelf life for different order reactions.

Reaction order	Model*	Shelf life (t _s)	Reaction constant units (k)
Zero	$[C] = [C_0] - k_0 t$	$t_s = \frac{C_0 - C}{k_0}$	(meq O ₂ /kg oil)/day
First	$\ln[C] = \ln[C_0] - k_1 t$	$t_s = \frac{\ln(\frac{C_0}{C})}{k_1}$	day ⁻¹
Second	$\frac{1}{[C]} = \frac{1}{[C_0]} + k_2 t$	$t_s = \frac{(\frac{1}{C}) - (\frac{1}{C_0})}{k_2}$	(meq O ₂ /kg oil) ⁻¹ .day ⁻¹

*C is the concentration of the monitored quality control indicators during the storage period (i.e., PV in meq O₂/[kg oil]& TVC in log₁₀ CFU/g), k is the reaction rate constant, n is the order of reaction (dimensionless), and t is the storage time (day).

3. Results and discussion

3.1. Interaction effect of propolis concentration and storage days on peroxide value of FFB

Auto-oxidation is a common chemical reaction in oil or fat-based products due to various agents during storage. Fat oxidation (formation of hydroperoxides) of unsaturated fatty

acids (USFA), particularly polyunsaturated fatty acids (PUFA), in frozen fish burgers (FFB) during storage directly affects product quality (19, 20). Peroxide value (PV) is the primary method for measuring lipid oxidation in FFB, with increases in this index leading to severe degradation of USFA (3). Fig. 1-A presents the mean comparison of PV values for FFB during 3 months of storage at -18°C. Results indicate that both the type of FFB (I, II, III & IV) and storage duration had

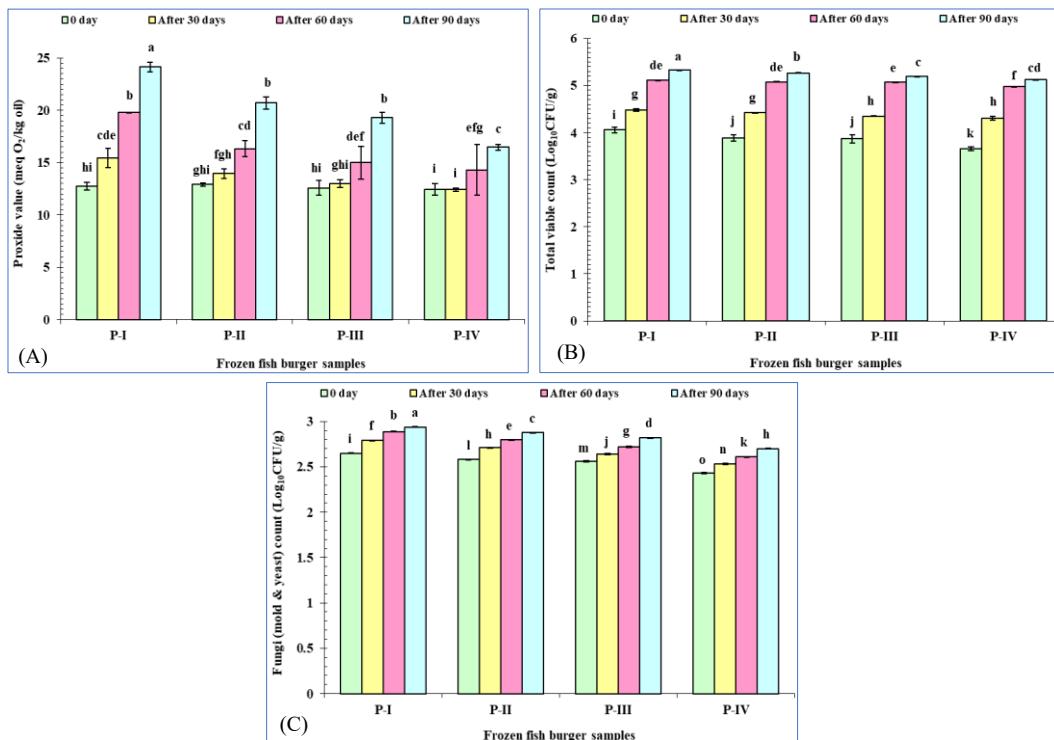


Fig. 1. The interaction effects of different propolis concentration (from zero to 0.4%) and storage days (up to 90 days) on the peroxide values (A), the total viable count (B) and the fungi (C) of FFB in frozen storage at -18°C.

significant effects ($p < 0.05$) on PV values. The lowest PV values throughout the storage period were observed in the P-IV sample (containing 0.4% propolis). All examined samples showed increased PV values as storage duration increased from 0 to 90 days. Previous studies have shown that adding natural plant-based additives (e.g., oregano, green tea, sage, and laurel) to ready-to-eat products like FFB reduces lipid oxidation progression (21, 22). According to Connell (23), acceptable PV for food consumption ranges between 10 to 20 meq O_2 /[kg oil]. Additionally, incorporating organic compounds (such as herbal extracts with high total phenolic content) into fish products significantly delays PV increases during storage.

3.2. Interaction effect of propolis concentration and storage days on the total viable count of FFB

Bacterial growth is the primary mode of product spoilage, and bacterial counts serve as an important quality indicator for fish burgers. Fig. 1-B illustrates the interactive effect of propolis concentration and storage duration on the total viable count (TVC) of FFB during three months of storage at -18°C . Results show that both FFB type (I, II, III & IV) and storage duration significantly affected ($p < 0.05$) TVC values. The lowest and highest TVC values were observed in P-IV (day 0 of storage) and P-I (after 90 days of storage), respectively. This trend is primarily attributed to the inhibitory effect of propolis, particularly the hydroxyl groups of phenolic compounds, on bacterial cell membrane activity through binding to cell walls (3). Similar findings were reported by Çoban and Keleştemur (24), who used *Zataria multiflora* Boiss essential oil (0.4% w/v) in catfish burger formulations. Their results showed significantly reduced microbial loads compared to control samples. All examined samples exhibited increased TVC values as storage duration increased from 0 to 90 days. Previous studies have demonstrated that adding natural plant-based additives (e.g., propolis, oregano, green tea, sage, and laurel) to ready-to-eat products like FFB reduces microbial growth (21, 22). According to food safety standards, acceptable TVC levels should be within certain limits to ensure product safety and quality. Furthermore, incorporating organic compounds (such as herbal extracts with high total phenolic content) into fish products considerably delays TVC increases during storage.

3.3. Interaction effect of propolis concentration and storage days on fungi of FFB

The ANOVA results showed that both the type of FFB (I, II, III & IV) and storage duration significantly affected ($p < 0.05$) the fungi (mold and yeast) count in FFB (Fig. 1-C). After 90 days of storage at -18°C , the control sample (P-I, without propolis) reached a fungal count of $2.94 \log_{10}$ CFU/g. In contrast, the lowest fungal count ($2.43 \log_{10}$ CFU/g) was observed in the P-IV sample (containing 0.4% propolis), representing an approximately 18% reduction compared to the control sample under similar conditions. These fungal counts were lower than the corresponding total viable count (TVC)

values, indicating a lower growth rate for fungi. This difference can be attributed to propolis's more effective antifungal properties than its antibacterial effects. Additionally, the meticulous nature of fungi and the abundance of nutritious compounds in FFB favor bacterial growth over fungal growth, resulting in higher TVC values (25). Notably, fungal growth in all FFB samples remained below the permissible value of $3 \log_{10}$ CFU/g set by the Iranian national standard throughout the entire storage period (26). This finding underscores the effectiveness of propolis as a natural preservative against fungal growth in frozen fish burgers. Özvural et al. (27) reported similar findings in hamburger patties stored at 4°C for 8 days, where adding 5% green tea extract significantly reduced mesophilic bacteria and yeast counts compared to control samples. Almuhayawi (28) highlighted the broad antimicrobial efficacy of propolis against bacteria, viruses, fungi, and protozoa, attributing this effect to compounds such as terpenoid luteol, flavonoids (fisetin, quercetin, pinocembrin, apigenin), and phenolic compounds (kaempferide, cinnamic acid).

3.4. Kinetics modeling of FFB's lipid and microbial degradation

To optimize storage conditions for FFB enriched with various concentrations of propolis (0 to 0.4%), the kinetic changes of quality control indicators were monitored in terms of chemical (fatty acid oxidation and monohydroperoxide formation or MHP) and microbial (total viable count or TVC) properties during three months of storage at -18°C . Table 2 presents the kinetic data for peroxide value and total viable count in FFB fitted to zero, first, and second-order reaction models. Results indicate that changes in MHP (or TVC) concentration in FFB during storage follow a zero-order kinetic reaction, as confirmed by the $[R^2/P]$ ratio. The degradation rate (k_0) of fatty acids ranged from 0.0462 to $0.1283 \text{ meq } O_2/(\text{kg oil}/\text{day})$, while the growth rate of TVC ranged from 0.0148 to $0.0170 \log_{10}$ CFU/g/day. These findings align with Quevedo et al. (6), who reported a kinetic rate of $0.009 \text{ meq } O_2/(\text{kg oil}/\text{day})$ for PV formation in frozen industrial burgers at -18°C using the Weibull model. The optimal storage time (shelf life) was calculated using the equation $t_s = (C_0 - C)/k_0$, where product quality characteristics (especially rancidity and microbial load) are preserved, and consumers receive the most nutritional benefits. Based on chemical (PV) and microbial (TVC) quality control indicators (QCIs) and considering the reaction constant (k_0) for the zero-order kinetic model, the shelf life of the best treatment (P-IV, containing 0.4% propolis) was determined to be approximately 86 days for both QCIs.

3.5. Sensory evaluation of fish burger patties (FFB)

The scores for appearance and organoleptic attributes of uncooked FFB with different concentrations of propolis after three months of storage at -18°C are presented in Table 3. The overall acceptance scores of FFB formulated with different levels of propolis concentration were significantly ($p < 0.05$)

Table 2. Kinetic parameters for FFB's lipid oxidation (or microbial growth rate) after three months at -18°C storage.

Products (^c ₁)	Zero order reaction					First order reaction					Second order reaction				
	k ₀	R ² ₀	P ₀ (%)	R ² ₀ /P ₀ (%) ^{(c} ₂)	C ₀ (meq O ₂ /kg oil)	k ₁	R ² ₁	P ₁ (%)	R ² ₁ /P ₁ (%)	C ₀ (meq O ₂ /kg oil)	k ₂	R ² ₂	P ² (%)	R ² ₂ /P ₂ (%)	C ₀ (meq O ₂ /kg oil)
Peroxide value															
P-I	0.1283	0.9892	0.0876	11.292	12.266	0.0072	0.9977	0.2627	3.7978	12.672	0.0004	0.9914	29.903	0.0331	12.903
P-II	0.0858	0.9227	0.0785	11.754	12.116	0.0052	0.9514	5.4914	0.1732	12.409	0.0003	0.9738	40.902	0.0238	12.610
P-III	0.0738	0.8653	0.2263	3.8237	11.647	0.0048	0.8930	0.6867	1.3004	11.909	0.0003	0.9174	19.831	0.0462	12.091
P-IV	0.0462	0.8866	0.1233	7.1906	11.851	0.0032	0.8950	4.4242	0.2023	11.955	0.0002	0.9011	32.385	0.0278	12.033
Total viable count															
P-I	0.0148	0.9686	0.150	6.457	4.080	0.0032	0.9643	0.681	1.416	4.093	-0.0007	0.9583	2.391	0.4008	4.103
P-II	0.0160	0.9607	0.150	6.407	3.949	0.0035	0.9523	0.073	13.04	3.961	-0.0008	0.9415	3.543	0.2657	3.968
P-III	0.0156	0.9418	0.000	0.000	3.918	0.0034	0.9374	1.200	0.781	3.928	-0.0008	0.9311	3.842	0.2423	3.937
P-IV	0.0170	0.9444	0.150	6.296	3.754	0.0039	0.9327	0.578	1.614	3.763	-0.0009	0.9181	1.147	0.8004	3.768

^{(c}₁) The Greece numbers (I), (II), (III), & (IV) represent the FFB containing different propolis concentrations (zero, 0.1, 0.2 & 0.4%), respectively. ^{(c}₂) The higher values of this ratio indicate the greater accuracy of the model in predicting the quality control indicators of FFB during storage.

Table 3. The scores of organoleptic attributes (^{(c}₁) of the FFB, including different amounts of propolis concentration after three months of storage at -18°C.

Products (^{(c} ₂)	General appearance	Aroma	Color	Texture	Total scores	Maximum possible score	Gaining scores out of maximum %
P-I	2.80±0.42 ^d	2.60±0.52 ^d	2.90±0.32 ^c	3.30±0.82 ^c	11.6±0.84 ^d	20	58
P-II	3.50±0.53 ^c	3.40±0.52 ^c	3.80±0.42 ^b	3.80±0.79 ^{bc}	14.5±0.85 ^c	20	72.5
P-III	4.30±0.48 ^b	4.20±0.42 ^b	4.70±0.48 ^a	4.20±0.42 ^{ab}	17.4±0.70 ^b	20	87
P-IV	4.90±0.32 ^a	4.70±0.48 ^a	4.90±0.32 ^a	4.70±0.48 ^a	19.2±0.79 ^a	20	96

^{(c}₁) Different superscript letters in each column indicate the significant (p<0.05) differences between the treatments. ^{(c}₂) The Greece numbers (I), (II), (III) & (IV) represent the FFB containing different propolis concentrations (zero, 0.1, 0.2 & 0.4%), respectively.

higher than the control samples (Table 3). While the control FFB sample (P-I) obtained 58% of the total possible sensory scores, those formulated with 0.1, 0.2, and 0.4% propolis gained approximately 72.5, 87, and 96% of the maximum scores, respectively. Furthermore, the ANOVA results confirmed that the FFB formulated with propolis (at different concentrations) exhibited significantly better general appearance, aroma, color, texture, and overall acceptance than those formulated without propolis (control samples).

3.6. Effects of propolis concentration on peroxide value, microbial activity, and overall acceptance of FFB during frozen storage

The incorporation of propolis influenced not only the chemical parameters (especially peroxide value, PV) and microbial activity (total viable count, TVC, and fungal count) but also the organoleptic properties (primarily appearance, aroma, color, and texture) of FFB during frozen storage. Moreover, as the propolis concentration in FFB increased (from 0% to 0.4%), the rate of PV production significantly diminished after three months of storage at -18°C (Fig. 2). A high and positive Pearson correlation ($r=+0.9969$ & $R^2=0.9938$) between PV and the reaction rate constant of the model (k_0) confirmed a strong and consistent dependence between these two parameters. Similarly, the TVC and fungal count production rate significantly decreased when the FFB samples were stored under the same conditions (Fig. 2). While the PV, TVC, and fungal count of the FFB sample containing 0.4% propolis reduced to approximately 32, 4, and 8.5%, respectively, after three months of storage at -18°C, its total acceptance scores for organoleptic properties increased by more than 65% compared to the control sample.

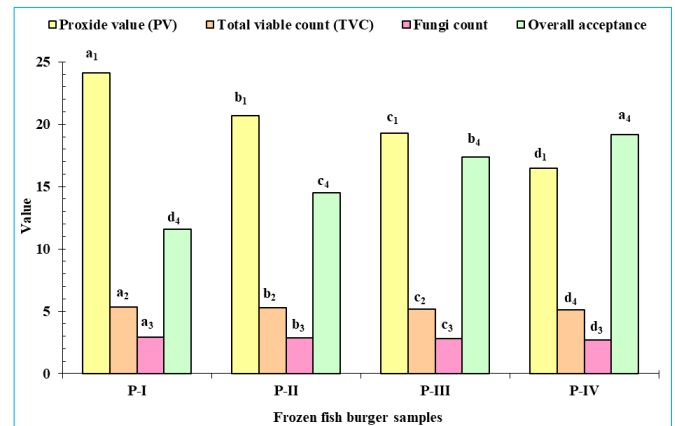


Fig. 2. The effects of fortifying FFB by adding propolis from zero (P-I) to 0.4% (P-IV) on chemical (PV in meq O₂/kg oil) and microbial (TVC and fungi, all in log₁₀CFU/g) properties along with their overall acceptance scores of organoleptic evaluations after three months storage (at -18°C).

4. Conclusion

This study demonstrates the potential of mathematical modeling to provide accurate and reliable predictions of fish freshness in fish burger patties and optimize food processes. The results indicated that the peroxide value and total viable count could estimate the shelf life of FFB when stored at -18°C. The frozen fish burger patties treated with 0.4% propolis powder (P-IV) and stored at -18°C for up to three months exhibited the lowest rate of lipid oxidation and microbial growth and received the highest overall acceptance scores. A zero-order kinetic model was used to evaluate the changes in PV and TVC during frozen storage. Additionally, the results

showed that the rate of change in PV (a primary oxidation product) was faster than the microbial attributes (approximately 32% for PV vs. 4% for TVC in the P-IV sample). In summary, rancidity is one of the first quality control indices to indicate the loss of quality in fish burger patties, and it can be controlled by reducing storage time and incorporating natural preservatives (at acceptable levels).

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