IMPACT OF PRESERVATION TECHNIQUES AND STORAGE DURATIONS ON NUTRITIONAL QUALITY OF ROHU AND RAINBOW TROUT

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Abstract: *Fish, a vital source of high-quality protein and essential nutrients, is highly perishable, necessitating effective preservation methods to maintain its nutritional value and ensure food security. This study assessed the effects of freezing (−20°C) and drying (60–70°C) over storage durations (0, 10, 20, 30 days) on the nutritional composition of Rohu (Labeo rohita) and Rainbow Trout (Oncorhynchus mykiss). Using a completely randomized design, key nutritional parameters—moisture, protein, crude fat, ash, minerals (calcium, iron, phosphorus, potassium, sodium), and vitamin C—were analyzed. Results showed freezing retained moisture and fat but caused protein and vitamin C degradation over time, while drying concentrated minerals like phosphorus and iron at the expense of moisture and vitamin C. Ash content increased under both methods due to mineral concentration from moisture loss. Nutrient-specific responses highlighted freezing's advantage in preserving lipid stability and drying's role in enhancing mineral density. Statistical analysis revealed significant interactions between preservation treatment and storage duration, influencing moisture, protein, iron, phosphorus, and potassium contents. Specifically, in Rohu, the interaction effects were statistically significant for moisture content $F(3,16) = 664$, $p < .001$, protein content $F(3,16) = 5.147$, $p < .01$ iron content $F(3,16) = 10.73$, $p < .001$, phosphorus content $F(3,16) = 68.08$, $p < .001$, and potassium content $F(3,16) = 5.801$, $p < .01$. A similar pattern was observed in Rainbow Trout, with significant interactions for the same nutrients. These findings elucidate the biochemical composition effects of preservation methods, offering practical strategies to optimize fish quality and sustainability. This research contributes to advancing sustainable aquaculture practices, improving food security, and promoting informed consumer choices.*

Keywords: Aquaculture; Biochemical Composition; Preservation; Shelf Life; Fish Quality; Nutrient Retention; Storage-induced

1. INTRODUCTION

Fish is globally recognized as an excellent source of animal protein and essential nutrients, playing a critical role in promoting human health and preventing various diseases (Abraha et al., 2018). Its diverse nutritional profile includes water (70–84%), protein (15–24%), fat (0.1–22%), minerals (1–2%), and small quantities of essential vitamins (Abraha et al. 2018). Fish contributes approximately 16% of the animal protein in the global diet and is a vital source of minerals and essential fatty acids (Pradeepkiran, 2019). Regular fish consumption has been linked to the prevention of high blood pressure, coronary heart disease, cancer, and inflammatory conditions, while also enhancing overall health (Abraha et al., 2018).

With rising global populations, the demand for fish is increasing due to its nutritional quality, appealing taste, efficient feed conversion, and commercial value (Pal et al., 2018). According to the Food and Agriculture Organization (FAO), global fish production stands at 167.2 million tons, of which 146.3 million tons are allocated for human consumption. The remaining portion is used for non-food purposes or discarded as waste. The high-quality nutrients in fish make it a primary

source of protein, while its harvesting, handling, processing, and distribution provide livelihoods for millions and contribute to foreign exchange earnings worldwide. However, fish is highly perishable, and spoilage often occurs due to enzymatic activity, lipid oxidation, and microbial growth, necessitating effective preservation techniques (Iheanacho, 2017).

In Bhutan, aquaculture was introduced in the early 1980s and has since evolved into a significant contributor to fish production. Recognized as a tool for achieving sustainable rural livelihoods, aquaculture has been supported by institutional reforms introduced in 2010. These reforms established the National Research and Development Center for Aquaculture (NRDCA), focusing on warm-water fish culture, and the National Research Center for Riverine and Lake Fishery (NRCRLF), focusing on cold-water fish culture (Wangchuk, 2019). With the growth of fish farming in Bhutan, educating the public about effective preservation methods is essential to ensure extended shelf life and sustained nutritional quality.

Fish preservation is crucial, particularly during peak harvesting seasons, to ensure a consistent supply throughout the year. Traditional preservation methods such as freezing and drying help reduce postharvest losses and improve shelf life, but their impact on the nutritional composition of fish remains uncertain (Sefa-Dedeh, 2011). While preservation techniques are widely practiced in Bhutan, there is limited awareness among consumers about the nutritional changes these methods may induce. As fish farming continues to expand in the country, understanding how preservation techniques affect nutritional value is essential for informed consumer choices and improved food security.
This study aims to address the knowledge gap by evaluating the individual and combined effects of freezing and drying on the nutritional composition of fish. Additionally, it seeks to determine the optimal storage duration for these methods to maximize nutrient retention. By providing insights into the best preservation practices, this research will contribute to the sustainability of Bhutan's aquaculture industry while ensuring that fish consumers continue to benefit from its nutritional value.

2. MATERIALS AND METHODS

This study investigated the effects of preservation methods, including freezing and drying, alongside varying storage durations, on the nutritional composition of fish. The research was conducted using the laboratory facilities at the College of Natural Resources (CNR) in Barp Gewog, Punakha Dzongkhag. Rainbow Trout, sourced from the National Coldwater Research and Fisheries Laboratory (NCR&LF) in Haa Dzongkhag, and Rohu, obtained from the National Research Development Centre for Aquaculture (NRDCA) in Sarpang Dzongkhag, were used as the fish samples for this study.
All animal care and use followed ethical standards, with approval from the institutional review board. The fish samples were handled with care to minimize stress during collection and processing. A Completely Randomized Design (CRD) was employed for the experimental setup. Fresh samples of Rohu and Rainbow Trout, each weighing between 400 and 1000 grams and measuring approximately 30 to 50 centimeters in length, were randomly collected. The fish were thoroughly washed with clean water to remove contaminants, and the non-edible portions (head, tail, fins and viscera) were removed to determine the percentage of edible portions.

Fish from both species were cut into pieces and divided into seven equal portions, each weighing 500 grams. These portions

consisted of one fresh sample, three samples designated for freezing, and three samples designated for oven drying from both the species. Each portion was stored for specific time periods (Fresh, 10, 20, and 30 days) and preserved using one of two methods: freezing at −20°C or oven drying at 60–70°C. The proximate analysis of the fresh sample from both species was conducted immediately after processing.

For the drying process, a 500-gram portion of each species was placed in a hot air oven to remove moisture, homogenized into a powdered form to ensure consistent nutrient distribution, and stored in zip-lock bags for laboratory analysis.

Proximate analysis was conducted to measure moisture content, protein, ash, lipid, calcium, phosphorus, sodium, potassium, vitamin C, and iron concentrations. The preservation and analytical processes were repeated at 10-day intervals throughout the 30-day storage period for both freezing and drying treatments. All measurements were performed in triplicate to reduce variability and enhance the accuracy of the results.

Data were recorded in Microsoft Excel and analyzed using R software. Normality tests were performed on the data before statistical analysis. The effects of freezing and drying over the 30-day storage period were analyzed separately for each species using one-way ANOVA, with significant differences within groups identified. To further assess the impact of preservation methods and storage durations on the nutritional composition of Rohu and Rainbow Trout, a two-way ANOVA was performed. Descriptive statistics, including mean and standard deviation, were used to present the results, ensuring clarity and precision in the interpretation of findings.

3. RESULTS AND DISCUSSIONS

3.1 Nutritional dynamics in preserved Rohu fish

3.1.1 Effect of Freezing and Drying Over 30 Days on the Chemical Composition of Rohu Fish using one-way Anova

The chemical composition of Rohu fish (*Labeo rohita*) underwent notable changes during storage under freezing and drying conditions for 30 days. The moisture content, an essential parameter for fish quality, decreased over time in both treatments. Fresh Rohu showed a mean moisture content of 73.64%, which declined to 73.1% after 10 days of freezing and further to 71.13% after 30 days. This slight reduction contrasts findings by Malik et al. (2021), who reported a consistent decrease in moisture with extended storage durations. The observed pattern may be attributed to myofibrillar distortion during freezing, impairing the muscles' water retention capacity, as described by Gandotra (2012). Conversely, drying proved effective in reducing moisture content more rapidly due to higher storage temperatures, a trend consistent with Yasin et al. (2016).

Protein content exhibited distinct trends under freezing and drying. In the fresh samples, the protein level was 11.17%, which steadily declined to 9.5% by day 30 under freezing conditions. This reduction aligns with studies by Aberoumand (2023) and Norrelykke et al. (2006), which associate protein losses during freezing with denaturation and chemical degradation. Similarly, Malik et al. (2021) reported decreased protein content in frozen fish over extended storage. In contrast, drying initially caused an increase in protein concentration from 11.17% on day 0 to 12.38% on day 10, likely due to water evaporation. However, protein levels subsequently declined to 10.21% at day 20 before stabilizing at

12.53% by day 30. These fluctuations may result from protein degradation due to enzymatic activity or exposure to drying temperatures. Tarle et al. (2016) reported no significant difference in protein content across drying methods for other fish species, indicating possible species-specific or methodological variability.

Crude fat levels remained largely unaffected during the 30-day storage under both freezing and drying, with no significant differences observed ($p > 0.05$) using one-way Anova. The suppression of enzymatic and oxidative processes at low storage temperatures likely preserved fat content. In contrast, ash content increased significantly $F(3, 8) = 6.36$, $p=.016$ in frozen samples by day 30 but showed no significant variation in dried samples. Okeyo et al. (2009) observed that ash content was unrelated to storage time, but the observed increase in frozen samples here may result from reduced moisture and protein levels, concentrating other components like minerals.

In essence, freezing and drying impacted the chemical composition of Rohu fish differently over 30 days. Freezing led to gradual declines in moisture and protein content, while drying initially increased protein concentration before a subsequent decline. Fat content remained stable across both treatments, whereas ash content increased in frozen samples. These findings provide insights into the storage-induced changes in fish composition, highlighting the influence of storage methods on the nutritional quality of Rohu fish and offering guidance for optimizing storage practices to preserve fish quality.

3.1.2 Effect of Freezing and Drying Over 30 Days on Mineral and Vitamin C Composition of Rohu using one-way ANOVA

The analysis of the mineral composition of Rohu under freezing and drying conditions over 30 days (Table 2) demonstrates varied impacts of these preservation methods. Freezing did not significantly affect calcium $F(3, 8) = 1.303$, $p = 0.339$, iron $F(3,8) = 5.065$, $p = 0.30$, sodium $F(3, 8) = 3.042$, $p = 0.093$, and potassium content $F(3, 8) = 3.354$, $p = 0.076$, corroborating findings by Malik et al. (2021), who observed similar trends in Bulti and Khasm elbanat fish. This stability likely reflects the minimal impact of freezing on the ionic integrity of these minerals. Wolfe and Bryant (2001) attributed minor mineral losses during freezing to the separation of water from the colloidal solution, forming pure ice. Moreover, the findings align with Sharaf (2013), who found no definitive relationship between freezing duration and mineral composition in Tilapia muscles stored at −18°C.

The calcium content remained consistent throughout the drying process, likely due to its primary association with bone, which remains unaffected by moisture loss. In contrast, potassium exhibited a notable fluctuation: an initial decline after 10 days of drying (5.77 mg compared to 7.67 mg in fresh samples), followed by an increase at 20 days (7.30 mg) and a peak at 30 days (9.50 mg). This pattern may be attributed to potassium redistribution within fish tissues during moisture loss and protein denaturation, resulting in localized concentration changes.

Phosphorus content in Rohu remained stable during the initial freezing period (10 days) but showed a significant increase $F(3, 8) = 905.175$, $p = 0.000$ after 20 days of storage. Jadhav and Magar (1970) similarly noted that phosphorus content in frozen samples remained unaffected in the short term. However, drying led to a marked increase in phosphorus concentration, likely due to moisture reduction concentrating the mineral. This observation aligns with the concentration effects reported in the study by Egun et al. (2023), suggesting that enzymatic

breakdown of phosphorus occurs more slowly under drying conditions, necessitating extended storage durations for noticeable changes. Iron content also increased significantly F (3, 8) = 60.786, p = .000 during drying, from a mean of 0.04% in fresh samples to 0.51% after 30 days. This increase is likely due to the removal of water, which concentrates the iron content within the fish tissue.

Vitamin C content showed a consistent decline with increased storage duration under both freezing and drying conditions. As a water-soluble and oxidation-sensitive compound, vitamin C is highly susceptible to degradation in the presence of light, oxygen, and enzymatic activity (Giannakourou & Taoukis, 2021). While freezing reduces enzymatic activity, it does not completely inhibit it, and trace oxygen in sealed packages contributes to gradual vitamin C loss. This aligns with Alahmad et al. (2021), who reported minimal variation in vitamin C levels in dried fish fillets. Drying, with its exposure to heat and air, exacerbates the degradation of vitamin C, confirming its instability under prolonged preservation.

Overall, these findings reveal that while freezing maintains the mineral integrity of Rohu, drying exerts significant concentration effects on certain minerals. Both methods, however, lead to a decline in vitamin C over time. These results are consistent with the tested hypotheses, emphasizing that the preservation processes impact the nutritional quality of Rohu in distinct ways. These observations can guide storage and processing methods to balance nutrient retention in fish products.

3.1.3 Interaction Effect between Storage Duration and Treatment on Various Nutritional Contents of Rohu Fish using two-way ANOVA

The results indicate that storage duration and treatment significantly ($p < 0.05$) influence certain nutritional components of Rohu fish. Figure 1 demonstrates the interaction between these factors, highlighting changes in moisture, protein, iron, potassium, and phosphorus contents. While these parameters showed variations, no statistically significant interaction effects between storage duration and treatment were observed for most of them. However, crude fat content was significantly affected by treatment, $F(1,16)=7.593$, $p<0.05$ $F(1,16) = 7.593$, \, $p < 0.05$ $F(1,16)=7.593$, $p<0.05$. Freezing for 20 days resulted in the highest crude fat content (20.67%), while drying for 30 days yielded the lowest (9.67%). This variation may be attributed to the lipid retention in frozen samples compared to oxidative degradation in dried samples over prolonged storage.

Table 1: Changes in nutritional content of Rohu fish over 30 days freezing and drying

Storage Durations	Method(s)	Duration			
		0 day	10 days	20 days	30 days
Moisture (%)	Freezing	73.64 ± 0.06^{a}	73.1 ± 0.17^{b}	73.05 ± 0.05^{d}	71.13± 0.13c
	Drying	73.64 ± 0.06^{a}	73.10 ± 0.11^{b}	72.93 ± 0.06^{c}	68.85 ± 0.02^{d}
Protein (%)	Freezing	11.17 ± 0.32^{a}	11.17 ± 0.32^{a}	9.54 ± 0.49^{b}	9.5 ± 0.96^{b}
	Drying	11.17 ± 0.3^{ab}	12.38 ± 0.4^{ab}	10.21 ± 1.4^{a}	12.53 ± 0.5^{a}
Crude fat (%)	Freezing	17.83 ± 2.48^{a}	17 ± 3.46^{a}	20.67 ± 0.58^{a}	19 ± 1.0^{a}
	Drying	17.83 ± 2.48^{a}	12.33 ± 2.89^{a}	9.67 ± 4.04^{a}	17.67 ± 9.29^{a}
Ash (%)	Freezing	20.67 ± 1.72^{a}	21.93 ± 1.55^{ab}	21.73 ± 4.61^{ab}	27.77 ± 0.32^{b}
	Drying	20.67 ± 1.72^{a}	24.67 ± 2.91^{a}	26.2 ± 8.23^{a}	22.5 ± 5.7^{a}

*Means with different superscript in the same row differed significantly ($p < .05$); SD

Ash and calcium contents exhibited no significant interaction effects, suggesting their relative stability across different storage and treatment conditions. However, storage duration had a significant effect on sodium content, F (3,16) = 3.393, $p < 0.05$, as did the treatment method, F (1,16) = 5.297, $p < 0.05$.

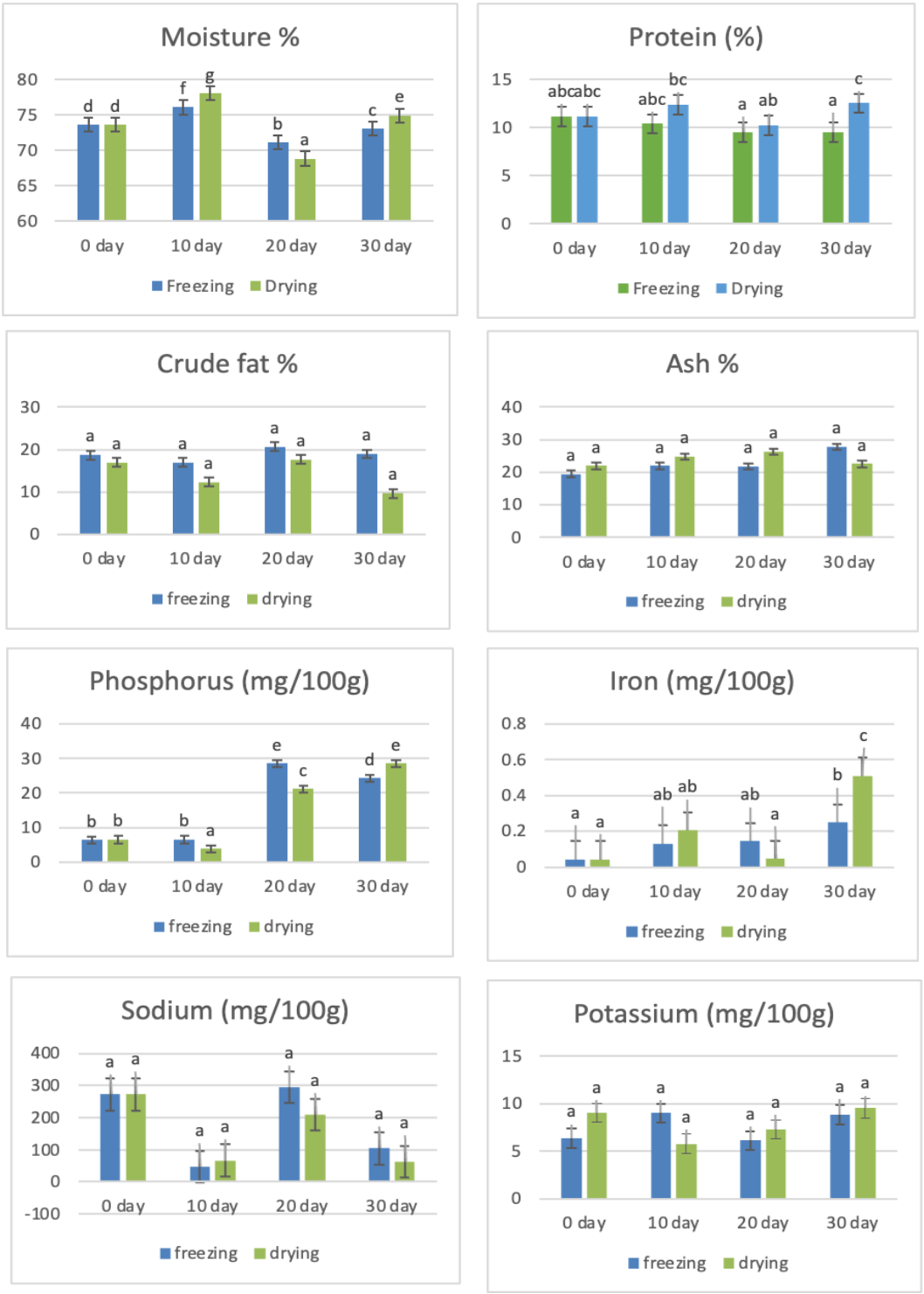


Figure 1: Changes in nutritional composition of Rohu fish subjected to freezing and drying preservation over 30 days with alphabetical superscripts representing significant differences and error bars indicating estimated errors in each measurement.

Notably, sodium content was highest (294.67 mg) at 20 days of freezing, while the lowest content (46.87 mg) was observed at 10 days. The absence of interaction effects between storage duration and treatment suggests that these factors contributed independently to sodium variations. These fluctuations may be attributed to differences in water loss during drying or sodium retention during freezing.

Vitamin C content did not show any significant effects from storage duration, treatment, or their interaction, indicating that the nutrient is unaffected under the tested conditions. This stability could be due to limited exposure to oxidative or thermal degradation.

Overall, the findings suggest that treatment methods, particularly freezing and drying, play a critical role in determining the nutritional quality of Rohu fish during storage. The data align with previous studies emphasizing the protective effects of freezing on lipid and mineral content while underscoring the susceptibility of certain nutrients, like fats, to degradation during drying. These insights contribute to optimizing preservation strategies to retain the nutritional value of Rohu fish, supporting both consumer health and post-harvest management practices.

3.2 Nutritional Composition in Preserved Rainbow Trout

3.2.1 Effect of Freezing and Drying Over 30 Days on Chemical Composition of Rainbow Trout

The study revealed significant variations in the chemical composition of Rainbow Trout subjected to freezing and drying over 30 days. Moisture content exhibited fluctuations influenced by storage conditions and drip loss during freezing. The oven drying process showed a gradual decrease in moisture, starting from 76.87% and reaching 74.85% by day 30, consistent with findings from Longwe and Kapute (2016), where similar reductions were observed in other fish species. This indicates that prolonged drying promotes moisture loss, which directly affects the texture and quality of fish.

Table 2: Changes in minerals and vitamin C content of Rohu over 30 days

Storage Durations	Method(s)	Duration			
		0 day	10 days	20 days	30 days
Calcium (mg/100g)	Freezing	12.75 ± 0.42^{a}	12.5 ± 0.5^{a}	12.33 ± 0.58^{a}	12.33 ± 0.58^{a}
	Drying	12.75 ± 0.42^{a}	13 ± 1^{a}	12 ± 0^{a}	12 ± 0^{a}
Iron (mg/100g)	Freezing	0.04 ± 0.02^{a}	0.13 ± 0.02^{a}	0.15 ± 0.01^{a}	0.25 ± 0.13^{a}
	Drying	0.04 ± 0.02^{a}	0.21 ± 0.05^{b}	0.05 ± 0.02^{a}	0.51 ± 0.08^{c}
Phosphorus (mg/100g)	Freezing	6.42 ± 0.85^{a}	6.49 ± 0.75^{a}	28.56 ± 0.12^{b}	24.27 ± 0.07^{c}
	Drying	6.42 ± 0.85^{a}	3.8 ± 1.25^{b}	21.18 ± 0.07^{c}	28.12 ± 0.01^{d}
Potassium (mg/100g)	Freezing	7.67 ± 1.95^{a}	9 ± 0.36^{a}	6.1 ± 1.61^{a}	8.8 ± 1.39^{a}
	Drying	7.67 ± 1.95^{a}	5.77 ± 1.72^{b}	7.3 ± 1.05^{ab}	9.5 ± 0.56^{a}
Sodium (mg/100g)	Freezing	272.1 ± 330^{a}	46.87 ± 15.4^{a}	294.67 ± 194^{a}	104.1 ± 75^{a}
	Drying	272.1 ± 330^{a}	65.9 ± 10^{ab}	209 ± 109.3^{b}	62.77 ± 10.3^{a}
Vitamin C (mg/100g)	Freezing	5.74 ± 1.89^{a}	4.29 ± 0.4^{a}	4.94 ± 0.17^{a}	3.13 ± 0.39^{a}
	Drying	5.74 ± 1.89^{a}	4.62 ± 0.7^{a}	5.24 ± 0.05^{a}	4.57 ± 0.38^{a}

**Means with different superscript in the same row differed significantly ($p < .05$); SD*

Protein content demonstrated distinct trends between freezing and drying. No significant changes occurred during the initial 10 days of freezing, but a significant increase was observed at day 20 (13.77%), followed by a decline to 9.48% by day 30. The initial rise may be attributed to water concentration during freezing, enhancing protein density temporarily. The subsequent decline suggests protein degradation due to prolonged freezing, as well as protein-lipid interactions. These results partially contrast with Beyrer (2006), who reported no significant impact of freezing on protein content in Rainbow Trout. For drying, protein content consistently decreased over time, aligning with Smida (2014), who observed similar declines in dried fish muscle due to protein denaturation under heat exposure.

Crude fat content remained stable throughout the 30-day freezing and drying periods, indicating that neither process significantly affected the lipid composition of Rainbow Trout. This stability is critical for maintaining the fish's nutritional quality during extended storage. Similarly, ash content increased during freezing and drying, attributed to the concentration of inorganic minerals as water content decreased. The findings align with Hussein et al. (2020), who reported rising ash content in frozen fish over prolonged storage periods. During drying, the increase in ash content suggests a proportional enhancement of mineral concentration as organic matter diminishes.It reveals that freezing and drying over 30 days result in noticeable changes in the chemical composition of Rainbow Trout. While moisture and protein content are significantly affected, crude fat remains unchanged, and ash content increases due to concentration effects. These findings provide valuable insights into the impact of storage methods on fish quality, offering a broader understanding of preservation techniques and their implications for food processing and storage.

3.2.2 Effect of Freezing and Drying Over 30 Days of Storage on the Mineral and Vitamin C Composition of Rainbow Trout

The study evaluated the impact of freezing and drying on the mineral and vitamin C content of rainbow trout over a 30-day storage period, revealing notable trends in nutrient retention and loss under these preservation methods. The results (Table 4) highlight that calcium content in frozen trout showed minimal variation over the storage period, remaining relatively stable compared to its fresh state, which aligns with findings by Anene (2015) indicating that freezing has a negligible effect on calcium retention. Conversely, during drying, calcium content remained consistent, suggesting that drying might effectively preserve this mineral.

Iron content exhibited little change during freezing until day 30, when a notable increase was observed, potentially linked to tissue changes caused by prolonged storage. Jamila (2020) reported similar fluctuations in iron levels during frozen storage, which may arise from structural disruptions within the fish tissue. Phosphorus levels in frozen samples initially decreased by day 10 but increased significantly F (3, 80) = 207.882, p = .000 by days 20 and 30, likely due to the physical effects of ice crystal formation. Drying led to a marked increase in phosphorus content after 20 and 30 days, attributed to moisture loss and the resulting concentration effect.

Potassium content in frozen trout increased significantly by day 20, followed by a slight decrease at day 30, possibly reflecting cellular changes within the muscle tissue during freezing.

Table 3: Changes in Nutritional Content of Rainbow Trout over 30 days of freezing and drying preservation method (Mean ± SD)

Storage Durations	Method(s)	Duration			
		0 day	10 days	20 days	30 days
Moisture (%)	Freezing	76.87 ± 0.1^{ab}	76.57 ± 0.49^{b}	74.76 ± 0.05^{b}	75.24 ± 0.22^{c}
	Drying	76.87 ± 0.1^{a}	76.83 ± 0.15^{a}	75.98 ± 0.1^{a}	74.85 ± 0.06^{b}
Protein (%)	Freezing	11.31 ± 1^{ab}	11.11 ± 1.36^{ab}	13.77 ± 1.15^{b}	9.48 ± 0.5^{a}
	Drying	11.31 ± 1^{a}	10.77 ± 1.1^{a}	10.23 ± 0.5^{a}	9.31 ± 2.4^{a}
Crude fat (%)	Freezing	27.17 ± 2.04^{a}	28.33 ± 2.31^{a}	26.67 ± 2.08^{a}	25.67 ± 3.51^{a}
	Drying	27.17 ± 2.04^{a}	26.67 ± 0.58^{a}	25.67 ± 1.53^{a}	19 ± 6.93^{a}
Ash (%)	Freezing	17.97 ± 2.23^{a}	19.93 ± 0.9^{a}	19.67 ± 0.76^{a}	21.74 ± 5.85^{a}
	Drying	17.97 ± 2.23^{a}	20 ± 1.71^{a}	21.47 ± 0.12^{a}	26.5 ± 3.18^{b}

**Means with different superscript in the same row differed significantly ($p < .05$); SD*

These findings correspond with Mphande and Chama (2021), who observed varied mineral retention in frozen fish species over time. Drying caused a significant rise in potassium content by day 30, reinforcing the concentration effect associated with moisture reduction. Sodium levels showed a substantial increase during freezing, peaking at day 20, while drying induced a temporary decrease at day 10, followed by a sharp increase at day 20, potentially linked to protein breakdown and the release of bound sodium.

Vitamin C content decreased progressively during both freezing and drying, consistent with findings by Sahari et al. (2013) and Dabrowski et al. (1988), which reported significant reductions in ascorbic acid levels during storage due to oxidative degradation. This decline underscores the susceptibility of vitamin C to preservation methods, particularly in prolonged storage. These results demonstrate the nuanced effects of freezing and drying on the nutritional composition of rainbow trout, with each method presenting unique advantages and limitations. Freezing preserved calcium and phosphorus effectively, while drying enhanced mineral concentration due to moisture loss. However, both methods led to a decline in vitamin C content. These findings highlight the need for tailored preservation strategies to optimize nutritional outcomes based on specific dietary requirements and storage goals.

3.2.3 Interaction Effect Between Storage Duration and Treatment on Various Nutritional Contents of Rainbow Trout Fish

The study revealed significant interaction effects between storage duration and treatment on various nutritional contents of Rainbow trout. Moisture content, iron content, phosphorus, potassium, and vitamin C exhibited notable differences, with estimated errors for each measurement represented by error bars in Figure 2. These findings underscore the dynamic response of the fish's nutritional composition to storage and treatment.

Protein content was significantly influenced by storage period (F (3,16) = 4.480, $p < .01$), with no significant effects for treatment or the interaction between storage period and treatment. Protein levels were highest at 20 days of freezing (13.77%) and lowest at 30 days of drying (9.31%), indicating that prolonged drying leads to substantial protein loss, potentially due to oxidative denaturation.

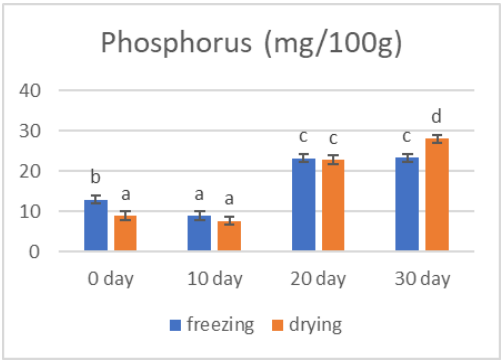
Table 4: Changes in minerals and vitamin C content of Rainbow trout over 30 days freezing and drying

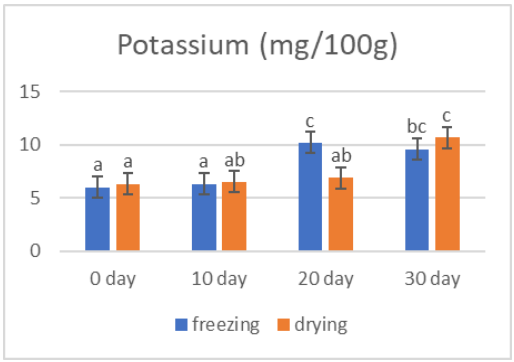
Storage Durations	Method(s)	Duration			
		0 day	10 days	20 days	30 days
Calcium (mg/100g)	Freezing	13.25 ± 0.42^{a}	13.5 ± 0.5^{a}	11.33 ± 0.58^{b}	13 ± 1^{a}
	Drying	13.25 ± 0.42^{a}	13.5 ± 0.5^{a}	12.67 ± 0.58^{a}	12.67 ± 1.15^{a}
Iron (mg/100g)	Freezing	0.07 ±0.04^{a}	0.06 ± 0.01^{a}	0.07 ± 0.01^{a}	0.38 ± 0.15^{b}
	Drying	0.07 ±0.04^{a}	0.07 ± 0.07^{a}	0.18 ± 0.02^{ab}	0.23 ± 0.01^{b}
Phosphorus (mg/100g)	Freezing	10.88 ± 2.46^{a}	8.87 ± 1.73^{b}	23.14 ± 0.14^{c}	23.26 ± 0.05^{c}
	Drying	10.88 ± 2.46^{a}	7.61 ± 1.15^{a}	22.8 ± 0.01^{b}	27.94 ± 0.04^{c}
Potassium (mg/100g)	Freezing	6.17 ± 0.93^{a}	6.33 ± 1.33^{a}	10.2 ± 0.66^{b}	9.6 ± 1.15^{b}
	Drying	6.17 ± 0.93^{a}	6.5 ± 1.3^{a}	6.9 ± 1.04^{a}	10.7 ± 0.36^{b}
Sodium (mg/100g)	Freezing	152.88± 76^{ab}	92.77± 57^{a}	500.67 ± 215^{b}	179.6± 101^{ab}
	Drying	152.88 ± 76^{a}	108.67 ± 1^{a}	500.67 ± 215^{b}	57.03 ± 20.42^{a}
Vitamin C (mg/100g)	Freezing	5.63 ± 0.19^{a}	5.96 ± 0.43^{a}	3.42 ± 0.16^{b}	2.81 ± 0^{b}
	Drying	5.63 ± 0.19^{a}	5.39 ± 0.55^{a}	5.4 ± 0.05^{a}	3.45 ± 0.22^{b}

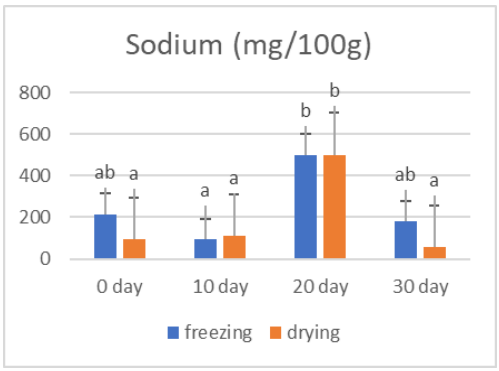
**Means with different superscript in the same row differed significantly ($p < .05$); SD*

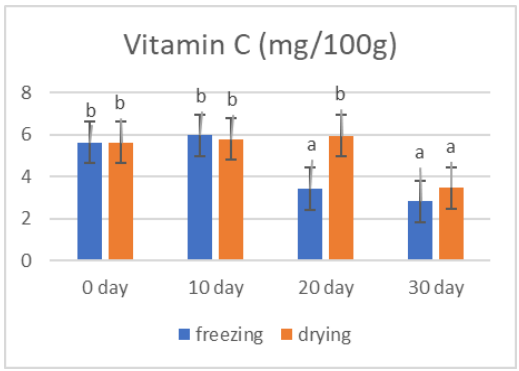
For crude fat, storage duration had a significant effect (F (3,16) = 3.425, p < .05), but treatment and interaction effects were not significant. Drying at 30 days retained the highest crude fat content (23.3%), while freezing at 10 days resulted in the lowest (19%). This variation might be attributed to lipid oxidation during drying, which is intensified over longer durations, thereby preserving lipids more effectively compared to freezing.

Calcium content was significantly affected by storage duration (F (3,16) = 5.533, p < .01). The highest calcium content (13.5 mg) was observed at 10 days of drying, while the lowest (11.3 mg) occurred at 20 days









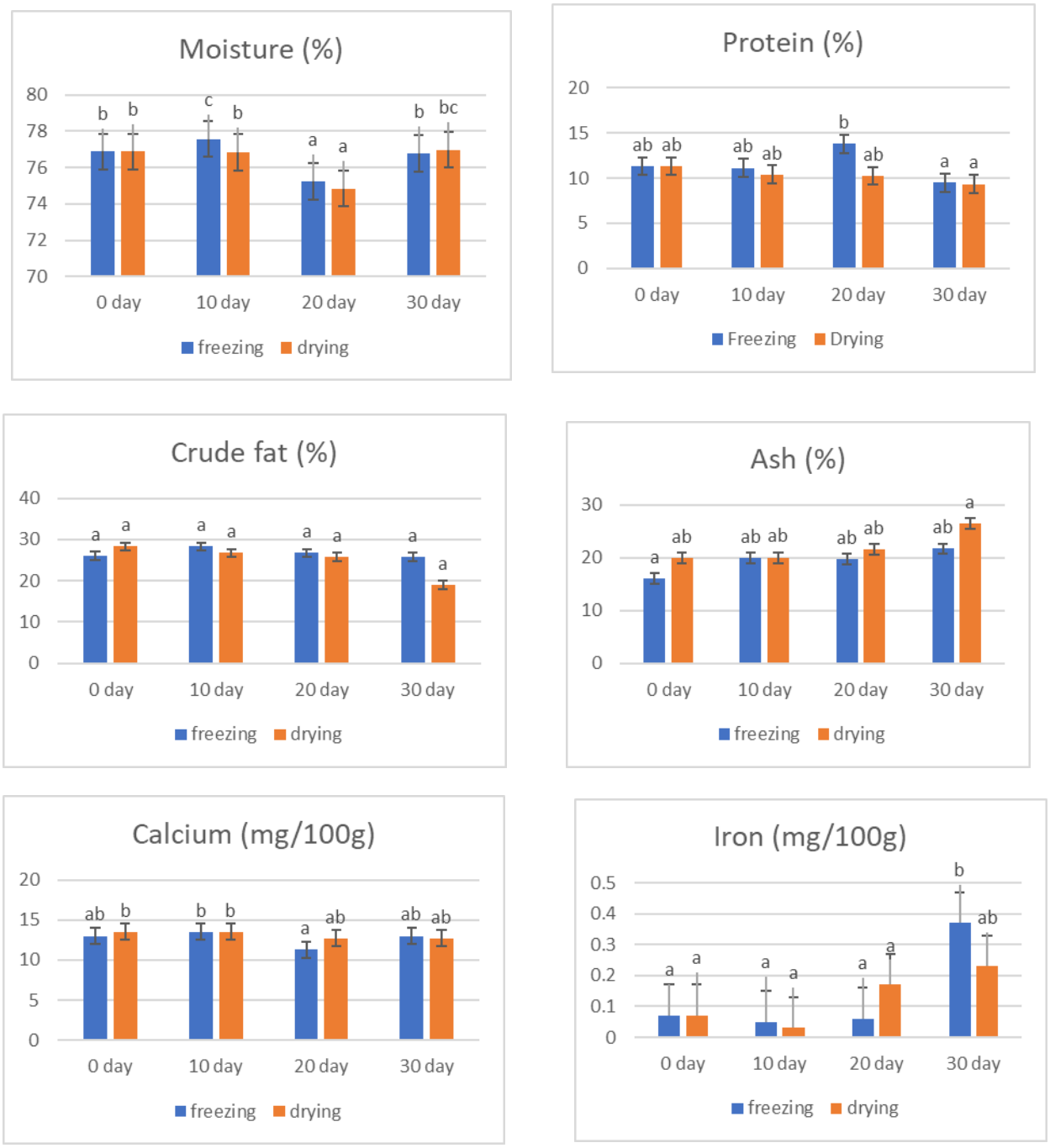


Figure 2: Nutritional composition changes in Rainbow trout over 30 days of freezing and drying, with superscripts denoting significant differences and error bars showing measurement errors

of freezing. The lack of significant treatment effects or interaction suggests that calcium levels primarily depend on storage time rather than the preservation method, likely due to mineral stability under different environmental conditions. Ash content demonstrated significant effects of both storage duration (F (3,16) = 6.374, p < .01) and treatment (F (3,16) = 6.760, p < .05), but no interaction effects were noted. Drying for 30 days yielded the highest ash content (26.5%), whereas freezing fresh samples resulted in the lowest (16%). This could be linked to the evaporation of water during drying, concentrating the mineral residues.

Sodium content varied significantly across storage durations, with dried fish at 20 days containing the highest sodium (500.7 mg), while 30 days of drying showed the lowest (57 mg). However, treatment and interaction effects were not statistically significant. These results suggest that prolonged drying leads to sodium leaching, likely due to osmotic shifts and water loss.

These findings align with previous studies demonstrating how storage and preservation methods impact the biochemical composition of fish. The significant changes in protein, crude fat, calcium, and ash content highlight the importance of selecting appropriate storage durations and treatments to optimize the nutritional quality of Rainbow trout. Although the hypotheses regarding interaction effects on all nutrients were not fully supported, the observed variations provide critical insights into the biological mechanisms underlying nutrient stability under different storage conditions.

4. CONCLUSION

This study explored the effects of freezing and drying preservation methods over 30 days on the nutritional composition of Rohu and Rainbow Trout, with an emphasis on key nutrients such as moisture, protein, fat, ash, minerals, and vitamin C. The findings revealed distinct impacts of these preservation techniques on the nutritional quality of fish, providing valuable insights for optimizing storage practices to maintain their nutritional integrity.

Freezing proved effective in preserving fat and maintaining the stability of minerals like calcium and sodium, with only minimal nutrient losses over time. However, protein levels exhibited a gradual decline during extended freezing, likely due to chemical denaturation and enzymatic degradation. Drying, on the other hand, effectively concentrated minerals such as phosphorus and iron due to moisture reduction but also resulted in fluctuations in protein content. Vitamin C, a highly sensitive nutrient, experienced consistent degradation under both preservation methods, underscoring its vulnerability to oxidative and thermal stress.

The study highlighted significant interactions between storage duration and preservation methods on various nutritional parameters, including moisture, protein, fat, and mineral content. For Rohu, freezing was more effective in maintaining nutrient stability over time, whereas drying enhanced mineral concentrations but led to greater nutrient degradation. Similarly, Rainbow Trout demonstrated that freezing preserved crude fat effectively, while drying increased ash content through concentration effects.

In conclusion, this research emphasizes the need to tailor preservation methods to specific nutritional priorities. Freezing is recommended for preserving protein and fat integrity over longer storage durations, while drying is advantageous for concentrating minerals. Both methods, however, require careful management of storage duration to minimize nutrient degradation, particularly for sensitive compounds like vitamin C. These findings serve as a basis for enhancing fish preservation practices in aquaculture industry, promoting better consumer health and supporting sustainable fishery management.

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