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Effects of Refrigeration and Freezing on Nitrate, Nitrite, and Oxalate Levels in Green Beans and Peas

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Abstract

This study investigates the effect of the blanching, refrigeration, and freezing on nitrate, nitrite, and oxalate levels in green beans and peas. Besides, the impact of these preservation methods on the nutritional and safety aspects of these vegetables was studied. The data revealed that the refrigeration significantly impacts nitrate levels in both green beans and peas, potentially due to biochemical and metabolic processes. Furthermore, there was a notable decrease in nitrate content from 2121.17 mg.kg-1 to 1371.74 mg.kg-1 in green beans after blanching and six months of freezing storage. This reduction in nitrate levels is attributed to both blanching and freezing storage, potentially influenced by enzymatic activities and chemical reactions. The study also observed a decline in oxalate levels, suggesting that blanching and freezing storage contribute to reduced oxalate content, likely influenced by enzymatic and chemical reactions. These findings align with previous research indicating significant reductions in nitrates, nitrites, and oxalates due to blanching, particularly for leafy vegetables, reinforcing the observed trends during freezing storage. In essence, the cold storage, blanching, and freezing processes led to reduced levels of nitrate, nitrite, and oxalates in green beans and peas.

Keywords; beans, peas, nitrate, nitrite, oxalate, cold storage, freezing.

INTRODUCTION

The nitrate, nitrite, and oxalate content of green beans and peas is an important area of study due to its potential impact on human health and food safety. Nitrate is a natural component of many vegetables, including green beans and peas, but high levels of nitrate can be of concern, particularly for infants and pregnant women due to the potential conversion of nitrate to nitrite and the associated health risks (Wu et al., 2021). Similarly, high oxalate content in these vegetables can contribute to the formation of kidney stones in susceptible individuals. Understanding the levels and effects of these compounds in green beans and peas is crucial for promoting their consumption as part of a healthy diet while mitigating potential health risks (Santamaria et al., 1999). Additionally, investigating the impact of storage, cooking, and processing on these compounds is essential for ensuring the nutritional and safety aspects of these popular legumes (Salehzadeh et al., 2020). The impact of cold storage on the nitrate, nitrite, and oxalate levels of green beans and peas is a significant area of study with implications for food safety and nutritional quality. Research findings suggest that blanching, freezing, and extended storage duration can lead to changes in the contents of these compounds in various vegetables, including spinach and dill. Specifically, blanching has been observed to induce a considerable reduction in the levels of nitrates, nitrites, and oxalates in leafy vegetables (Kmiecik et al., 2004). Furthermore, freezing and storage of frozen products have shown varying effects on the content of nitrates and nitrites in vegetables such as peas. Understanding these changes and their implications is crucial for ensuring the nutritional value and safety of green beans and peas during storage, as well as for establishing best practices for preserving their quality (Jaworska, 2005). The objectives of this study are (i) to investigate the impact of blanching, cold storage, and freezing on of nitrate, nitrite, and oxalate levels in green beans and peas and to comprehend how these preservation methods affect the nutritional and safety aspects of these vegetables; (ii) to discern the influence of these processes on the biochemical and metabolic pathways leading to changes in nitrate and oxalate content.

MATERIALS AND METHODS

Materials:

Green beans (*Phaseolus vulgaris* L.*)*, and Peas (*Pisum sativum* L.) samples were obtained from local farm closed to in Sohag, Egypt.

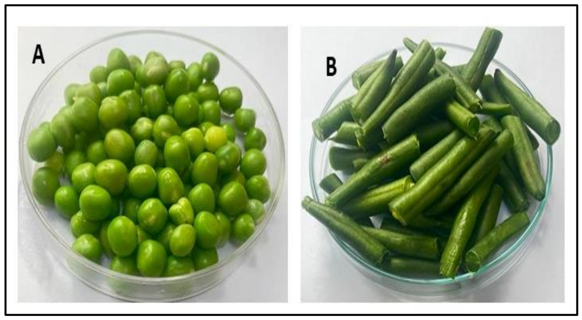

Figure 1. Green bean (A) and green pea (B)

Chemicals and reagents:

Aqueous methanol, Folin-Ciocalteu and gallic acid were obtained from Sigma (Germany). The other chemicals were of the highest quality was purchased from El-Gomhoria Company, Egypt.

Preparation of samples:

The green bean and pea samples underwent a thorough washing with fresh water, the peas were trimmed into small pieces and the seeds were extracted for beans. After this, the samples were divided for different treatments:

1. Some samples were kept fresh and refrigerated at 4°C for several days until they were considered inedible.
2. Others were blanched at 95°C for 5 minutes and then frozen at -20°C using a deep freezer for 6 months. These samples were utilized for chemical analysis every month.

Analytical methods:

Preparation of extracts

Fresh and frozen samples were handled differently before undergoing extraction using the Rousseaux et al. (2005) method. Two-gram samples were mixed with 5 ml of ammonia buffer solution (pH 9.6-9.7), 50 ml of alumina cream, and 50 ml of distilled water. The mixture was then agitated for 5 minutes using a Waring Blender. Subsequently, distilled water was added up to 200 ml before filtration through Whatman No. 4 filter paper. The resulting filtrate was then analyzed for nitrite and nitrate levels.

Nitrate and nitrite determination

The nitrite content was assessed calorimetrically following the procedure outlined by Abu-Dayeh et al. (2007). A standard curve was developed using sodium nitrite solution. For nitrate content determination, nitrate was reduced to nitrite using spongy metallic cadmium, and then assessed following the previously mentioned method. The reduction and assessment were conducted as per the method detailed by Cortesi et al. (2015).

Oxalates determination

The samples were dried in an oven at 45ºC for 72 hours, ground into a fine powder, packed in polyethylene bags, and stored at -4ºC until required for analysis. 0.1g of the powder sample was weighed into a 100ml conical flask. Subsequently, 75ml of $3MH_2SO_4$ was added and stirred for 1 hour using a magnetic stirrer. After filtration with a Whatman No 1 filter paper, 25ml of the filtrate was taken and titrated while hot against a 0.05 M $KMnO_4$ solution until a faint pink color persisted for at least 30 seconds. The oxalate content was calculated using 1 ml of 0.05 M KMnO4 as equivalent to 2.2 mg oxalate, as per Chinma and Igyor (2007).

Statistical analysis

Data were statistically analyzed by SAS statistical software (SAS ver. 9.2, SAS Institute, 2008).

RESULTS AND DISCUSSION

Effect of cold storage on nitrate, nitrite, and oxalate content of green beans

The data presented on Table (1) shows that the nitrate content of green beans during cold storage gradually decrease as the duration of storage increases. From the initial level of 2252.58 $mg.kg^{-1}$ in fresh green beans, there was a consistent decline in nitrate content over the 12-day storage period. to 1981.68 $mg.kg^{-1}$. This trend suggests that cold storage has a significant impact on the nitrate levels of green beans, with the observed reduction possibly attributed to biochemical and metabolic processes that occur during storage as reported by Ekart et al. (2013) and Balan et al. (2016). Further research and analysis could provide insights into the specific mechanisms behind this reduction and its implications for the nutritional quality and safety of green beans during cold storage as indicated by Armesto et al. (2017). The data (Table 1) provided indicates the nitrite content of fresh green beans condition and during cold storage for various time periods. Initially, the nitrite content was 90.30 ppm in fresh green beans. Subsequently, during the cold storage period, the nitrite levels fluctuated, with a decrease to 57.37 $mg.kg^{-1}$ at day 6, followed by an increase to 94.61 $mg.kg^{-1}$ at day 12. This fluctuation in nitrite content during cold storage suggests that the storage conditions may have a variable impact on the nitrite levels in green beans as mentioned by Semida, et al. (2023). Further analysis and research are required to understand the mechanisms behind these fluctuations and to determine the optimal storage conditions for preserving the nitrite content and overall quality of green beans as reported by Chung et al. (2004) and Silalahi et al. (2016).

Table 1. Effect of cold storage on nitrate, nitrite, and oxalate content of green beans

Component	Fresh Samples	Cold Storage (days) Samples					
		2	4	6	8	10	12
Nitrate ($mg.kg^{-1}$)	2252.58	2216.75	2198.69	2151.62	2078.78	2012.46	1981.68
Nitrite ($mg.kg^{-1}$)	90.30	87.00	57.37	68.47	90.38	94.01	94.61
Oxalate ($mg.g^{-1}$)	27.86	27.50	26.03	25.45	24.20	23.10	22.73

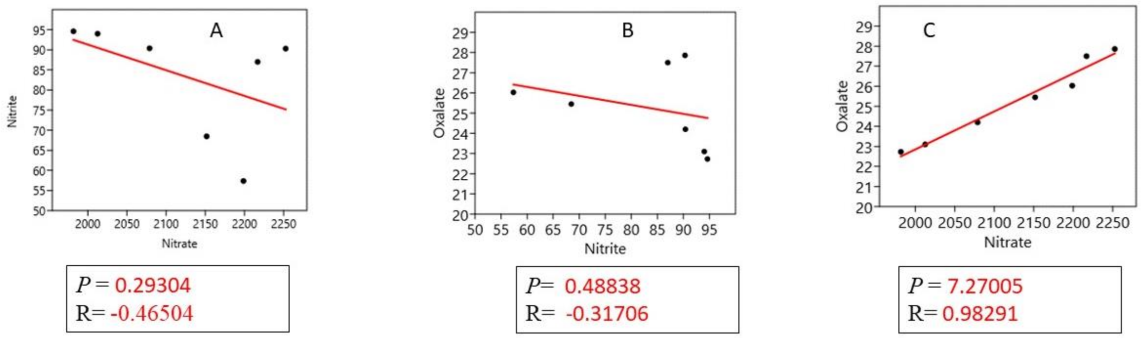

Figure 2. The regression curves between the studied components (nitrate, nitrite, oxalate) of green bean under cold storage treatments

Data in Table 1 also illustrates the impact of cold storage on the oxalate content of green beans over a period of 12 days. From these data, it can be observed that the oxalate content of fresh green beans (27.86 $mg.g^{-1}$) was gradually decreases as the duration of cold storage increases. However, the oxalate content decreased up to 22.73 $mg.g^{-1}$ of cold at the end storage (12 days). The obtained results revealed that the prolonged storage at lower temperatures (4°C) may lead to a reduction in oxalate content in green beans as cleared by Salgado et al. (2023). Further analysis and research into the mechanisms behind this reduction could provide valuable insights for optimizing the storage conditions and preserving the nutritional quality of green beans as reported by Monreal et al. (1999) and Hasan (2023). Figure (2) indicates that under cold storage treatment for green bean, a negative correlation was observed between nitrite and nitrate levels, as well as between nitrite and oxalate levels. This suggests that as levels of nitrite increase, levels of nitrate and oxalate decrease, and vice versa. Conversely, a positive correlation was noted between nitrate and oxalate levels, implying that as nitrate levels increase, oxalate levels also tend to increase. The findings could have implications for understanding the impact of cold storage treatment on the interaction and dynamics of these components in a given system. Further analysis and specific context are necessary to fully interpret and apply these results effectively as cleared by Jaworska (2005); Merusi et al. (2010); Wu et al. (2021) and Sorour et al. (2021).

Effect of freezing storage on nitrate, nitrite, and oxalate content of green beans

The given data in Table (2) presents the impact of blanching and subsequent freezing storage on the nitrate content of green beans over a period of six months. The data shows that after blanching, the nitrate levels decrease from 2121.17 $mg.kg^{-1}$ to 1371.74 $mg.kg^{-1}$ at the end of the freezing storage (6 months). This decline suggests that both blanching and freezing storage contribute to the reduction in nitrate content in green beans over time. The observed decrease in nitrate levels during freezing storage could be due to various factors such as enzymatic activities and chemical reactions that occur during storage as reported by Brown (1967) and Kaack (1994). Further analysis and research would provide valuable insights into the specific mechanisms behind this reduction and its implications for the nutritional value of green beans during freezing storage as mentioned by Leszczyńska et al. (2009). The presented data (Table 2) also illustrates the impact of blanching and subsequent freezing storage on the nitrite content of green beans over a span of six months. The nitrite levels exhibit fluctuations during the freezing storage period ware: 78.50 $mg.kg^{-1}$ after blanching, followed by 52.56, 34.59, 32.21, 44.41, and 53.29 $mg.kg^{-1}$ at 2, 3, 4, 5, and 6 months, respectively. These data indicates that the blanching and freezing storage processes significantly affect the nitrite content of green beans. Further analysis and research would be instrumental in comprehending the observed fluctuations and their implications for the nutritional quality and safety of green beans during freezing storage as reported by Drake et al. (1981) and Kmiecik et al. (2004).

Table 2. Effect of freezing storage on nitrate, nitrite, and oxalate content of green beans

Component	Blanched Samples	Freeze Storage (month) Samples					
		1	2	3	4	5	6
Nitrate ($mg.kg^{-1}$)	2121.17	2108.39	2096.23	1564.78	1560.34	1518.30	1371.74
Nitrite ($mg.kg^{-1}$)	78.50	52.56	34.59	32.21	44.41	53.29	84.38
Oxalate ($mg.g^{-1}$)	26.25	25.74	24.64	24.05	23.10	20.97	19.43

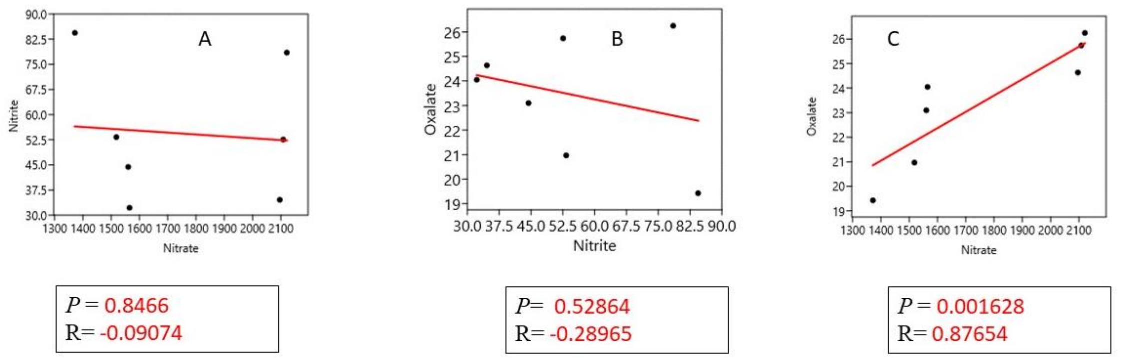

Figure 3. The regression curves between the studied components (nitrate, nitrite, oxalate) of green bean under freezing storage treatments

The provided data illustrates the impact of blanching and freezing storage on the oxalate content of green beans over a period of six months. The oxalate levels show a consistent decrease as the duration of freezing storage increases, ranging from 26.25 $mg.g^{-1}$ after blanching to 19.43 $mg.g^{-1}$ at the end of the sixth month. This decline suggests that both blanching and freezing storage contribute to the reduction in oxalate content in green beans over time. The observed decrease in oxalate levels during freezing storage could be attributed to enzymatic and chemical reactions that occur during the storage period as reported by Kmiecik et al. (2004). It is important to note that the findings of Sorour et al. (2021) which also suggests that blanching induced a considerable reduction in the contents of nitrates, nitrites, and oxalates, specifically for leafy vegetables. This information aligns with the observed trends in the oxalate content of green beans during the freezing storage period. Further research and analysis could provide valuable insights into the mechanisms behind this reduction and its implications for the nutritional quality and safety of green beans during freezing storage as cleared by Leszczyńska et al. (2009). In the context of cold storage treatment for green pea, Figure (3) demonstrates a negative correlation between nitrite and nitrate levels, as well as nitrite and oxalate levels. This signifies that as nitrite levels increase, nitrate and oxalate levels decrease, and vice versa. Conversely, a positive correlation is evident between nitrate and oxalate levels, indicating that as nitrate levels increase, oxalate levels also tend to increase. These findings could have implications for understanding the impact of cold storage treatment on the interaction and dynamics of these components within a given system. Further analysis and specific context are necessary for a comprehensive interpretation and practical application of these results (Jaworska, 2005; Merusi et al., 2010; Wu et al., 2021 and Sorour et al., 2021).

Effect of cold storage on nitrate, nitrite, and oxalate content of pea

The given data (Table 3) indicates the impact of cold storage on the nitrate content of peas over a storage period of 12 days. The nitrate levels appear to fluctuate during the cold storage period, starting at 1345.68 $mg.kg^{-1}$ in fresh peas and subsequently varying as follows: 1388.62, 1380.62, 1281.14, 1159.75, 1173.66, and 1140.09 mg.kg^{-1} after 2,4, 6, 8, 10 and 12 days, respectively. The trends in the nitrate content during the cold storage period warrant further investigation to understand the underlying mechanisms causing the fluctuations. Additional research and analysis could provide valuable insights into the impact of cold storage on the nitrate levels of peas and its implications for the nutritional quality and safety of the stored produce as reported by Huarte-Mendicoa et al. (1997) and Van der Sman (2020). The provided data demonstrates the impact of cold storage on the nitrite content of peas over a storage period of 12 days. The nitrite levels exhibit fluctuations during the cold storage period, starting at 33.75 mg.kg^{-1} in the fresh peas and subsequently varying over the storage duration. The nitrite content recorded: 33.01, 22.48, 38.73, 40.46, 39.77, and 36.72 mg.kg^{-1} after 2, 4, 6, 8, 10, and 12 days of storage, respectively. These fluctuations in nitrite content during cold storage suggest a varying impact of the storage conditions on the nitrite levels in peas. The increase observed on day 8 to a level of 4046 ppm may indicate a potential anomaly in the data. Further research and analysis are necessary to comprehend the mechanisms behind these fluctuations and their implications for the nutritional quality and safety of peas during cold storage. Understanding these variations is crucial for ensuring the preservation of nutritional quality in stored produce as cleared by Roszczenko et al. (2001) and Kmiecik et al. (2004).

Table 3. Effect of cold storage on nitrate, nitrite, and oxalate content of pea

Component	Fresh Samples	Cold Storage (days) Samples					
		2	4	6	8	10	12
Nitrate (mg.kg^{-1})	1345.68	1388.62	1380.62	1281.14	1159.75	1173.66	114.09
Nitrite (mg.kg^{-1})	33.75	33.01	22.48	38.73	4046	39.77	36.72
Oxalate (mg.g^{-1})	16.57	14.74	13.57	9.82	13.13	11.36	10.56

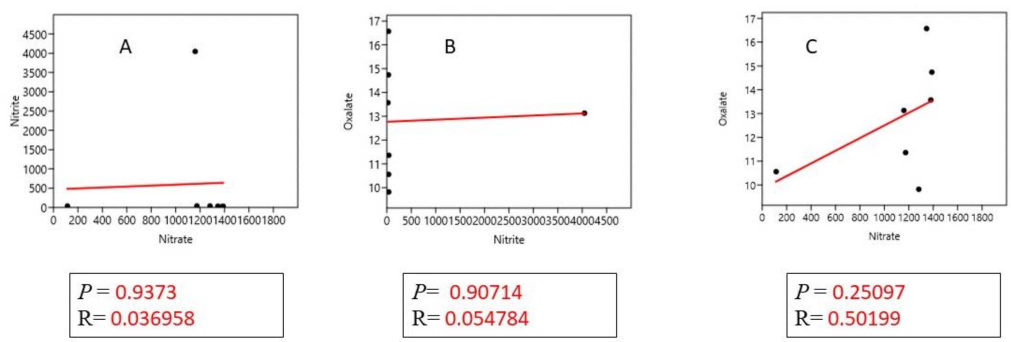

Figure 4. The regression curves between the studied components (nitrate, nitrite, oxalate) of green pea under cold storage treatments

The provided data (Table 3) showcases the effect of cold storage on the oxalate content of peas over a period of 12 days. The oxalate levels exhibit fluctuations during the cold storage periods, showing a decrease from 16.57 mg.g^{-1} in the fresh peas to 10.56 mg.g^{-1}by day 12 of cold storage. Notably, there was a slight decrease in oxalate content between day 2 and day 6, followed by a noticeable drop by day 8, with a subsequent minor increase by day 10. This fluctuation in oxalate content in peas during the cold storage periods could potentially signify the impact of storage conditions on the oxalate levels. Additional analysis and research may offer insights into the factors influencing these fluctuations, which could be crucial in understanding the nutritional changes that occur during cold storage as reported by Forni (1991) and Swieca et al. (2019). The results from Figure (4) highlight a significant positive correlation among all the studied components, namely nitrite and nitrate, nitrite and oxalate, as well as nitrate and oxalate, under freezing conditions for duration of up to six months at approximately -20 °C for green bean. This indicates that as the levels of one component decrease, the levels of the others also tend to decrease, suggesting a cohesive relationship among these compounds under these specific freezing conditions. These findings are valuable for understanding the behavior of the components during extended freezing periods and could inform various fields such as food storage and preservation (Kmiecik et al., 2004 and Sorour et al., 2021).

Effect of freeze storage on nitrate, nitrite, and oxalate content of pea

Data in Table 4 provided showcases the impact of blanching and subsequent freezing storage on the nitrate content of peas over a period of six months. After blanching, the nitrate levels decreased progressively during the freezing storage period, from 1152.34 mg.kg^{-1} at the initial time to 595.71 mg.kg^{-1} at the end of the storage period. This information indicates a consistent reduction in nitrate content during freezing storage, which could be attributed to various factors such as enzymatic and chemical reactions that occur during the storage period. The observed decrease in nitrate levels underscores the importance of appropriate freezing techniques to maintain the nutritional quality of peas during long-term storage. This data underscores the significance of controlled freezing rates to preserve the nutritional content of vegetables during the freezing process as reported by Kaack (1994) and Leszczyńska et al. (2009). Data in Table 4 also illustrates the impact of blanching and subsequent freezing storage on the nitrite content of peas over a period of six months. The nitrite levels display variation during the freezing storage period, starting at 15.92 mg.kg^{-1}, reaching 0.00 mg.kg^{-1} from month 2 to 3, and then subsequently increasing to 42.93 mg.kg^{-1} after the sixth month. This fluctuation in nitrite content during freezing storage underscores the potential impact of storage conditions on the nitrite levels in peas. Further research and analysis may shed light on the mechanisms behind these fluctuations and their implications for the nutritional quality and safety of peas during freezing storage (Drake et al., 1981 and Kmiecik et al., 2004). Results in Table 4 demonstrate the effect of blanching and freezing storage on the oxalate content of peas over a period of six months. The oxalate levels showcase a gradual decrease throughout the freezing storage period, starting at 13.05 mg.g^{-1} after blanching and reaching 8.36 mg.g^{-1} by the end of the sixth month. This observed decrease in oxalate content during freezing storage may be attributed to various factors such as enzymatic and chemical reactions that occur during storage, as reported by Korus et al. (2011) on the effects of blanching and freezing on oxalate content of vegetables. This decrease in oxalate levels highlights the potential impact of freezing storage on the nutritional quality of peas. Further research and analysis could provide valuable insights into the mechanisms behind this reduction and its implications for the nutritional value and safety of peas during freezing storage as reported by Chai et al. (2005).

Table 4. Effect of freeze storage on nitrate, nitrite, and oxalate content of pea

Component	Blanched Samples	Freeze Storage (month) Samples					
		1	2	3	4	5	6
Nitrate (mg.kg^{-1})	1152.34	1005.19	780.17	787.87	643.97	605.19	595.71
Nitrite (mg.kg^{-1})	15.92	0.00	0.00	17.77	14.00	34.05	42.93
Oxalate (mg.g^{-1})	13.05	11.29	10.78	9.97	10.78	9.17	8.36

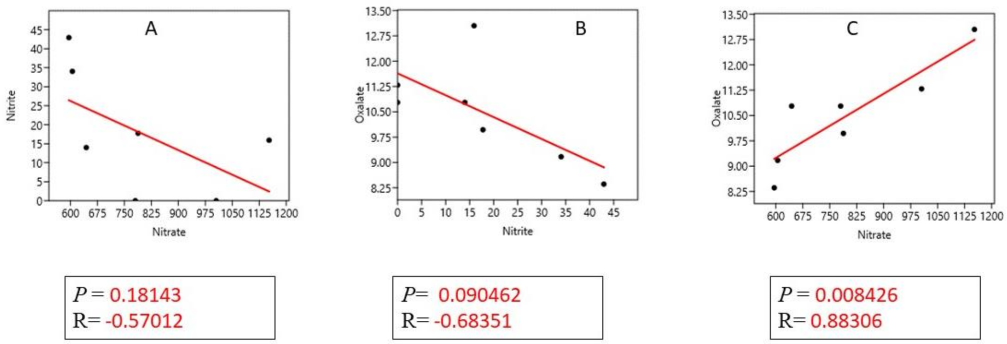

Figure 5. The regression curves between the studied components (nitrate, nitrite, oxalate) of green pea under freezing storage treatments

The data from Figure (5) for green peas during freezing over six months at approximately -20°C indicates a negative correlation between nitrite and nitrate, as well as nitrite and oxalate. In contrast, a positive correlation was observed between nitrate and oxalate. This suggests that when nitrite levels decrease, nitrate and oxalate levels tend to increase, and vice versa. Additionally, when nitrate levels elevate, oxalate levels also appear to increase. These findings offer valuable insights into the behavior of these components during extended freezing periods and could have implications for various practical applications, including food storage and preservation (Kmiecik et al., 2004 and Sorour et al., 2021).

CONCLUSION

In conclusion, this study demonstrates the significant impact of blanching, cold storage, and freezing processes on the levels of nitrate, nitrite, and oxalate in green beans and peas, shedding light on the effects of these preservation methods on the nutritional and safety aspects of these vegetables. The observed decrease in nitrate levels during cold storage and after blanching followed by freezing storage underscores the effectiveness of these processes in reducing nitrate content, likely influenced by enzymatic activities and chemical reactions. Additionally, the decline in oxalate levels further emphasizes the potential benefits of blanching and freezing storage in mitigating oxalate content. These findings align with existing research on reductions in nitrates, nitrites, and oxalates due to blanching, particularly for leafy vegetables, reinforcing the impact of these preservation methods. Overall, this study provides valuable insights into optimizing storage and processing techniques to minimize the levels of potentially harmful compounds in green beans and peas, contributing to enhanced food safety and quality.

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