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STUDY ON IMPACT OF DIFFERENT STORAGE TEMPERATURE ON THE GLUCOSE OXIDASE ACTIVITY OF ROYAL JELLY

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Abstract: A significant nutritional excretion of honeybees, royal jelly may have health benefits for us. This study examined at how the enzyme Glucose Oxidase changed following two years of frozen and refrigerated storage of Royal Jelly. After six months, refrigeration dramatically decreased glucose oxidase activity, whereas frozen samples showed no alterations. The findings imply that after two years of refrigeration, this enzyme activity may be a good indicator of the freshness of royal jelly. In comparison to refrigeration, freezing may better protect glucose oxidase for at least a year. Overall, freezing might be the best way to preserve the freshness and enzymatic integrity of Royal Jelly.

Keywords: Royal Jelly, Glucose Oxidase, frozen storage, freezing

1. Introduction:

The hypo-pharyngeal and mandibular glands of worker honeybees secrete royal jelly, a natural milky substance that serves as a full source of nutrition for honeybee larvae [1]. Proteins, lipids, carbs, vitamins, and minerals are among the several nutritional components found in royal jelly (2). Numerous qualities include anti-tumour and anti-inflammatory effects, anti-fatigue and hypotensive activity, antioxidant activities, antibacterial effects, and immunological activity enhancing. Royal jelly began to be quite valuable for humans because of its distinctive properties (3).

60–70% of RJ is made up of moisture, followed by 9–18% proteins, 7–18% carbs, and 3–8% lipids [4]. The primary amino acid is aspartic acid, and the primary fatty acid is 10-hydroxy-2-decenoic acid [5]. Over 90% of the total sugar content in RJ is made up of glucose and fructose [6].

Since their abundance may change according on storage conditions, time, and temperature, glucose oxidase was proposed as potential markers for RJ freshness [7, 8]. However, after a year of storage at room temperature, glucose oxidase was shown to be absent in RJ according to a proteomic analysis [9]. The enzyme

glucose oxidase, which is released by the hypo pharyngeal glands of bees, converts D-glucose into gluconic acid and hydrogen peroxide, the latter of which has antibacterial effects [10].

RJ is primarily produced by the hypo pharyngeal glands, and glucose oxidase has been found in their proteome [11]. Nectar also contains glucose oxidase [12, 13]. Therefore, it is challenging to determine whether this enzyme is synthesized by the bee and secreted in RJ or whether it originates from a plant.

However, the antibacterial and antioxidant effects of RJ might be impacted by the presence of glucose oxidase enzyme activity [14].For these reasons, the enzymatic activity measurement in RJ may be a useful method for evaluating the quality of a product during its shelf life. The study's objective was to examine the enzymatic activity of glucose oxidase in RJ during various storage conditions, including deep freezing at -18^0 and refrigeration at 4^0 Celsius.

2. Materials and Methods

2.1 Samples

The investigation was carried out on 4 samples of RJ for year produced in 2017(March), 2017(September), and 2018. Samples collected in 2021 represented the control of each enzymatic investigation. For each year of time, samples were collected from the same beekeepers, produced in the same geographical areas and analysed at the same time, belonging to the same commercial batches. These samples were stored at 4 ◦C (refrigeration, R) and at −18 ◦C (deep-freezing, F) until lab processing to analysis. [15].

2.2 Statistical analysis

All Values were expressed as means± SEM. Graph pad PRISM 8 was used for statistical analysis of the results. Data were analysed using the one-way analysis of variance (ANOVA) followed by Turkey's post hoc test for multiple comparisons. The value of ***P<0.001, **P<0.01, *P<0.05 were considered to be statistically significant.

3. Result

Table 1: Statistical differences in Glucose oxidase enzymatic activity in RJ samples refrigerated (R) at 4 ◦C and frozen (F) at −18 ◦C for different time interval.

Enzyme	**Time → Temp. ↓**	**2 months**	**6 months**	**1 years**	**2 years**
Glucose oxidase (U/mg proteins)	refrigerated 4 ◦C (R)	264.2 ± 19.14	142.8 ± 17.30	64.00 ± 4.25**	100 ± 9.125*
	frozen storage −18 ◦C (F)	257.7 ± 35.00	226.3 ± 28.69	344.7 ± 16.69	351.7 ± 23.10

P<0.05*, P<0.01**, P<0.001*** vs. 2 month

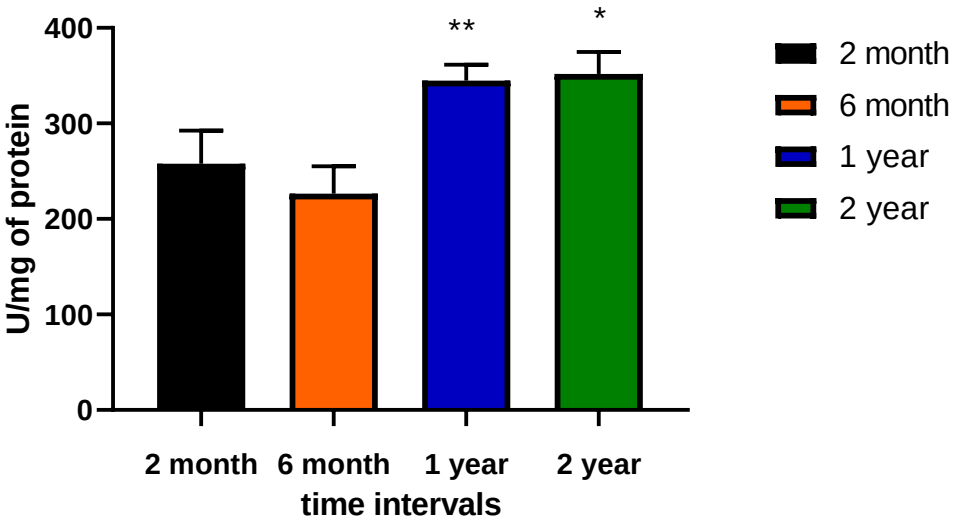

Fig1: Statistical differences in Glucose oxidase enzymatic activity in RJ samples refrigerated (R) at 4 ◦C

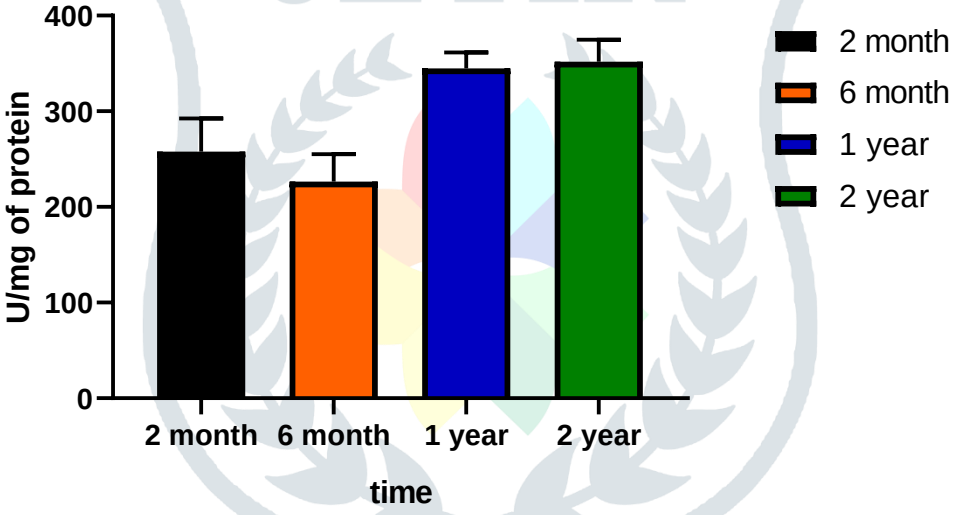

Fig2: Statistical differences in Glucose oxidase enzymatic activity in RJ samples frozen (F) at −18 ◦C for different time interval.

Glucose oxidase activity in refrigerated royal jelly (RJ) was significantly higher in 2 months of storage sample compared to 1and 2 years of storage. (Table 1).

A significant decline were recorded in 1 year ($P<0.01$) Refrigerated at 4^0C sample was seen when compared to 2 months storage sample.

A significant decrease in 2 year ($P<0.05$) Refrigerated at 4^0C sample was observed when compared to 2 months storage sample.

No significant differences were seen in glucose oxidase activity in 6 month storage samples compared to 2 month storage sample.

Non-significant increase were observed in 1 and 2 year samples of frozen stored at -18^0C compared to two month frozen storage royal jelly.

After 1 year refrigerated storage, glucose oxidase activity was lower than in samples stored frozen for 1 year. (Fig 1 & 2).

4. Discussion

This investigation focused on the temporal changes in glucose oxidase activity in royal jelly caused by freezing and refrigeration. After six months of refrigeration at 4°C, glucose oxidase activity considerably decreased but it then stabilized over the following years under the same conditions. The enzyme may be degrading or being inhibited by compounds like glucono-lactone if activity levels are declining. Because glucose oxidase can oxidize and break down the lipids in royal jelly, the reduction is significant. [18].

Lipid peroxidation is a known result of hydrogen peroxide produced by glucose oxidase [19]. However, its generation of hydrogen peroxide and antibacterial properties could potentially help stop degradation. [20]

After a year, frozen royal jelly still had more intense glucose oxidase activity than refrigerated royal jelly, however the activity had slightly decreased. Overall, the results indicate to significant declines in glucose oxidase activity within the first six months of storage as a potential freshness indication for royal jelly.

The results emphasize the need for focused study during the first year with shorter sample intervals to comprehend enzyme stability. Freezing helps maintain higher enzyme activity versus refrigeration alone.

5. Conclusion

Based on the results, it appears that glucose oxidase may be an accurate indicator of freshness in Royal Jelly, particularly within six months of production, as their activity showed a constant pattern regardless of the storage method used. Given that deep freezing specifies a high preservation of glucose oxidase after six months of storage, the results obtained further suggest that deep freezing may be a better alternative storage strategy to refrigeration.

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