APIMONDIA SCANDINAVIA 2025
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PP-231 [Beekeeping Technology and Quality]

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NEW POTENTIAL FRESHNESS MARKERS FOR ROYAL JELLY: GLUCOSE OXIDASE AND CARBOXYPEPTIDASE-A-LIKE

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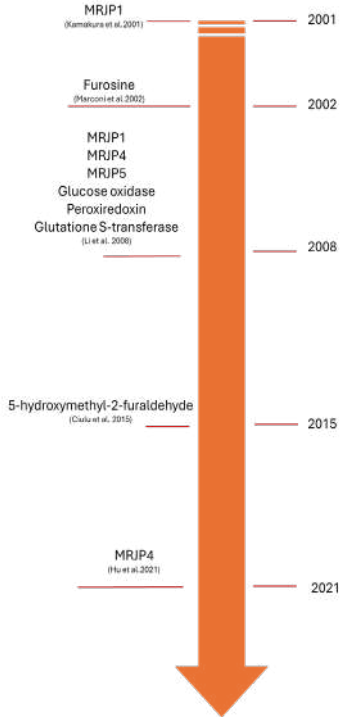
Several potential markers for freshness assessment have been tested and suggested in the last decades. However, implication of enzymatic activity involvement in RJ quality assessment during shelf life has been poorly investigated despite it can have potential affect on antimicrobial and antioxidant properties as well as in proteins degradation resulting in a loss of product quality



	RJ Storage Time – Refrigetation condition					
Enzyme	2m	1y	2y	3y	4y	*p-Value*
Glucose oxidase (U/mg proteins)	274 ± 70 [a] (279)	62 ± 21 [b] (45)	91 ± 6 [b] (91)	129 ± 24 [b] (130)	93 ± 30 [b] (98)	*0.015*
Carboxypeptidase-like A (U/mg proteins)	33.7 ± 10.5 [a] (22.8)	3.9 ± 1.3 [c] (5.2)	13.3 ± 2.3 [b] (13.1)	4.5 ± 1.4 [c] (4.3)	6.2 ± 3.2 [bc] (1.6)	*0.007*
Trypsin-like (U/mg proteins)	0.40 ± 0.02 (0.40)	0.50 ± 0.03 (0.48)	0.45 ± 0.03 (0.41)	0.47 ± 0.02 (0.47)	0.49 ± 0.05 (0.49)	*0.136*
Chymotrypsin-like (U/mg proteins)	0.07 ± 0.02 (0.06)	0.03 ± 0.01 (0.01)	0.08 ± 0.02 (0.06)	0.09 ± 0.02 (0.10)	0.07 ± 0.02 (0.06)	*0.271*
N-aminopeptidase-like (U/mg proteins)	0.58 ± 0.19 (0.44)	0.35 ± 0.06 (0.34)	0.30 ± 0.03 (0.32)	0.33 ± 0.03 (0.30)	0.32 ± 0.04 (0.31)	*0.692*
Carboxypeptidase-like B (U/mg proteins)	0.29 ± 0.21 (0.04)	0.20 ± 0.12 (0.04)	0.37 ± 0.30 (0.02)	0.19 ± 0.15 (0.00)	0.77 ± 0.22 (0.59)	*0.206*

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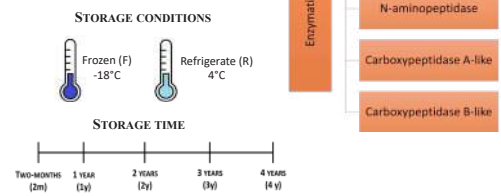
Enzyme	2mR *vs.* 1yR	2mF *vs.* 1yF	2mR *vs.* 2mF	1yR *vs.* 1yF
Glucose oxidase (U/mg proteins)	274 ± 70(279) *vs.* 62 ± 21(45) ***p = 0.009;***	772 ± 436(272) *vs.* 433 ± 140(392) *p = 0.754;*	274 ± 70(279) *vs.* 772 ± 436(272) *p = 0.754;*	62 ± 21(45) *vs.* 433 ± 140(392) ***p = 0.047;***
Carboxypeptidase A-like (U/mg proteins)	33.7 ± 10.5(22.8) *vs.* 3.9 ± 1.3(5.2) ***p = 0.006;***	9.4 ± 5.1(4.0) *vs.* 9.7 ± 3.4(7.5) *p = 0.584;*	33.7 ± 10.5(22.8) *vs.* 9.4 ± 5.1(4.0) ***p = 0.025;***	3.9 ± 1.3(5.2) *vs.* 9.7 ± 3.4(7.5) ***p = 0.047;***
Trypsin-like (U/ mg proteins)	0.40 ± 0.02(0.40) *vs.* 0.50 ± 0.03(0.48) ***p = 0.018;***	0.49 ± 0.04(0.46) *vs.* 0.50 ± 0.03(0.48) *p = 0.718;*	0.40 ± 0.02(0.40) *vs.* 0.49 ± 0.04(0.46) *p = 0.109;*	0.50 ± 0.03(0.48) *vs.* 0.50 ± 0.03(0.48) *p = 0.917;*
Chymotrypsin-like (U/mg proteins)	0.07 ± 0.02(0.06) *vs.* 0.03 ± 0.01(0.01) *p = 0.120;*	0.11 ± 0.02(0.11) *vs.* 0.05 ± 0.02(0.06) *p = 0.133;*	0.07 ± 0.02(0.06) *vs.* 0.11 ± 0.02(0.11) *p = 244;*	0.03 ± 0.01(0.01) *vs.* 0.05 ± 0.02(0.06) *p = 0.348;*
N-aminopeptidase-like (U/mg proteins)	0.58 ± 0.19(0.44) *vs.* 0.35 ± 0.06(0.34) *p = 0.361;*	0.43 ± 0.06(0.47) *vs.* 0.38 ± 0.08(0.28) *p = 0.465;*	0.58 ± 0.19(0.44) *vs.* 0.43 ± 0.06(0.47) *p = 0.749;*	0.35 ± 0.06(0.34) *vs.* 0.38 ± 0.08(0.28) *p = 0.917;*
Carboxypeptidase B-like (U/mg proteins)	0.29 ± 0.21(0.04) *vs.* 0.20 ± 0.12(0.04) *p = 0.848;*	0.13 ± 0.13(0.00) *vs.* 0.69 ± 0.65(0.00) *p = 0.416;*	0.29 ± 0.21(0.04) *vs.* 0.13 ± 0.13(0.00) *p = 0.295;*	0.20 ± 0.12(0.04) *vs.* 0.69 ± 0.65(0.00) *p = 0.738;*



Glucose oxidase and carboxypeptidase-A-like activity may be suitable markers of RJ freshness within one year at refrigeration condition
Freezing may be a valid alternative storage method to improve preservation of the activity of these enzymes.

DIPARTIMENTO DI SCIENZE VETERINARIE

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New potential freshness markers for Royal Jelly: Glucose oxidase and carboxypeptidase-A-like

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Royal Jelly (RJ) is a high value functional food in human nutrition due to its health-promoting properties. This has led to a growing interest by consumers and food industry towards this bee product so much so that several potential markers for freshness assessment have been tested and suggested. Implication of enzymatic activity involvement in RJ quality assessment during shelf life has been poorly investigated despite it can have potential affect on antimicrobial and antioxidant properties as well as in proteins degradation resulting in a loss of product quality. The aim of this study was to investigate the enzymatic activity of glucose oxidase (GOX) and five proteases (i.e. chymotrypsin, trypsin, N-aminopeptidase, carboxypeptidase A and B) in RJ under different storage condition (i.e. refrigeration at 4 °C and freezing at −18°) and times (i.e. Two months, one, two, three and for year of storage). Refrigeration determined a higher GOX activity in RJ after two months of storage that significantly decrease after one year. In refrigerated RJ samples also a higher carboxypeptidase-like A activity at two-months compared to other investigated time points was recorded suggesting a potential negative effect on RJ quality due to the proteolysis activity of this enzyme. Freezing did not determined variation in both GOX and carboxypeptidase-A-like activity in RJ samples stored for two months rather than after 1 year. However, frozen samples showed a higher GOX and carboxypeptidase-A-like activity after 1 year of storage compared to refrigeration. No variation in the activity of the other investigated proteases were recorded in both refrigerated and frozen samples. Therefore, the results obtained indicate that glucose oxidase and carboxypeptidase-A-like activity may be suitable markers of RJ freshness within one year at refrigeration condition. In addition, freezing may be a valid alternative storage method to improve preservation of the activity of these enzymes. However, further investigation to determine the timing of glucose oxidase inactivation/degradation under refrigerated conditions and the enzymatic activity trend under prolonged frozen conditions are desirable.