

Evolution of invertase activity in honey from *Castanea sativa* and *Rosmarinus officinalis* collected in Granada

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ABSTRACT

The content of α -glucosidase in two varieties of honey covered by the Granada Honey Protected Denomination of Origin Regulatory Board is studied. The evolution of invertase activity was followed for 10 months, in 12 monofloral chestnut honey samples collected in the Alpujarra area of Granada and 9 monofloral rosemary honey samples collected in la Resinera, Valle de Lecrin and in the Sierra de Baza for 10 months. The invertase was measured in the months of June 2005, September 2005, January 2006 and finally in the month of April 2006. We obtained an invertase activity in fresh chestnut honey of 194.6 ± 3.6 U/kg, with a range between 188.7 U/kg and 199.5 U/kg. After 10 months at ambient temperature, the chestnut honey lost between 28.3% and 39.8% of the initial values of α -glucosidase, with an average of $33.4 \pm 3.3\%$. In the rosemary honey we obtained average invertase values of 69.4 ± 14.9 U/kg, with a range between 56.9 U/kg and 91.9 U/kg. After 10 months at ambient temperature, the rosemary honey lost between 19.3% and 32.3% of the initial α -glucosidase values, with an average of $28.1 \pm 4.4\%$.

KEYWORDS: Honey, invertase, evolution, chestnut honey, rosemary honey, Granada, Spain.

1. INTRODUCTION

Honey is a complex substance that includes enzymes which contribute to its anti-bacterial properties. Invertase (α -glucosidase) is an enzyme that is produced in the hypopharyngeal glands and which is a catalyst for one of the most important reactions in the transformation of nectar (sucrose) into honey (glucose and fructose).

The content of invertase varies in relation to the botanical origin of the honey. It is known that some nectar require less manipulation by the bees in the hive to attain that thick consistency¹. It also depends on the age of the bee², the state of the colony³, the flow of nectar and the environmental conditions⁴.

The processing of honey before storage involves a series of manipulations, amongst which is the heating phase. Two parameters are measured normally to evaluate the freshness

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of the honey and any bad storage procedures, for example overheating, and these are diastase and hidroximetilfurfural (HMF) activity.

However, invertase is a parameter that is not normally considered for these ends, in spite of being more sensitive to heat, and it rather therefore a parameter to be used for measuring the quality of honey. White^{5,6} demonstrated that invertase was destroyed more quickly than amylase when honey was heated, so invertase activity could be a better indicator of honey quality than diastase activity. Dustmann⁷ states that invertase, in combination with other analytical criteria, is able to detect damage caused to the quality of the honey due to overheating or to long periods of storage.

In Spain, invertase is only used as a quality parameter by the Granada Honey Denomination of Origin Regulatory Board and by the Basque Label of Quality.

In Spain, the content of invertase in both fresh and industrially processed (heating by electric resistance and by pasteurisation) Spanish honey samples has been studied by diverse authors^{7,1}.

Sánchez⁸ studied the content of invertase in honey and its decline in time in samples collected in Galicia. They mainly studied thousand flower and eucalyptus honey (*Eucalyptus sp.*), and some blackberry (*Rubus sp.*) and chestnut (*Castanea sativa*).

The objective of this study was to study the evolution of invertase activity for a year in 2 monofloral honey samples from Granada under the umbrella of the Granada Honey Protected Denomination of Origin Regulatory Board. The aim was to understand its evolution and know the useful shelf life to guarantee the minimum contents established by the regulations.

2. MATERIAL AND METHODS

The evolution of invertase activity was followed for 10 months in 12 monofloral chestnut honey samples collected in the La Alpujarra area of Granada and 9 monofloral rosemary honey samples collected in la Resinera, Valle de Lecrin and in the Sierra de Baza, for 10 months.

The invertase was measured in the months of June 2005, September 2005, January 2006 and finally in the month of April 2006.

Invertase activity was determined according to the method of Siegenthaler⁹ based on the spectrophotometric measurement of 4-nitrophenol produced by the reaction of α -glucosidase with 4-nitrophenyl- α -D-glucopyranoside. The units are expressed in IU (International units U/kg)¹¹. Samples were maintained at $25 \pm 1^\circ\text{C}$.

The criteria used for the inclusion of the honey in the study were those used by the Granada Honey Protected Denomination of Origin Regulatory Board (Table 1). Melissopalynological analysis was performed using the methods recommended by the International Commission for Bee Botany¹², which involved 1200 pollen grains per sample.

Table 1. Minimum values established for some of the parameters in the regulations of the Granada Honey Protected Denomination of Origin

	Chestnut honey	Rosemary honey
Invertase	> 100 US	> 40 US
Colour	> 80 Pfund	> 35 Pfund
Conductivity	> 8 S/cm x 10 ⁻⁴	> 2,5 S/cm x 10 ⁻⁴
Percentage of pollen	> 75%	> 15% ó > 10% and presence >5% of other pollens from the family Labiatae.

3. RESULT AND DISCUSSION

We obtained invertase activity in fresh chestnut honey samples of 194.6±3.6 U/kg, with a range between 188.7 U/kg and 199.5 U/kg (Table 2). The range is very narrow with respect to other studies¹ as the samples come from the only chestnut area in Granada (Sierra Nevada – La Alpujarra). The average value of invertase is very similar to that obtained this author¹, who, in commercial chestnut honey samples obtained a value of 25.6±5.9 Gontarski units (SN: sucrose hydrolysed per 100gh⁻¹), which is equivalent to 190.5 U/kg in 5 samples of honey from different geographical areas of Spain with a range between (19.2 y 32.6 SN).

Table 2. Changes in Invertase Activity during 10 months in chestnut honey.

	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10	S 11	S 12	Meam ±SD
Jun05	196,3	198,8	197,1	196,5	188,7	195,3	190,5	192,3	195,6	195,3	199,5	189,3	194,6±3,6
Sep-05	161,2	155,8	156,7	165,3	177,1	167,2	156,3	156,3	157,8	158,3	164,3	175,9	162,7±7,2
Jan-06	159,7	157,5	143,5	163,4	155,4	150,9	139,6	152,6	145,3	144,9	161,9	154,1	152,4±7,7
Apr-06	134,3	127,6	118,6	131,1	133,3	132,4	130,2	127,9	129,6	120,6	132,9	135,8	129,5±5,3
Difference	61,9	71,2	78,5	65,3	55,3	62,9	60,3	64,4	66,0	74,7	66,6	53,5	65,1±7,3
Loss (%)	31,6	35,8	39,8	33,3	29,3	32,2	31,6	33,5	33,7	38,5	33,4	28,3	33,4±3,3

S = Sample

The initial values in the 2 chestnut Honey samples coming from Galicia are appreciably higher than our results (239.8 UI and 249.6 UI)⁹.

After 10 months at ambient temperature, the chestnut Honey lost between 28.3% and 39.8% of the initial values of α -glucosidase, with an average of 33.4%±3.3%. We found similar values in chestnut honey samples from Galicia at 12 months, 21.9% and 32.6%⁹.

In the Rosemary Honey, we obtained average invertase values of 69.4±14.9 U/kg, with a range between 56.9 U/kg and 91.9 U/kg. (Table n° 3). As we see in this case, the variability is greater as the samples were collected from 3 areas that were very different from the climactic and orographic point of view.

Table 3. Changes in Invertase Activity during 10 months in rosemary honey.

	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	Means±SD
Jun-05	56,9	91,9	60,1	60,3	90,9	59,8	59,8	60,5	84,3	69,4±14,9
Sep-05	53,4	75,6	54,8	55,8	74,9	55,6	54,1	55,7	69,1	61,0± 9,4
Jan-06	52,1	65,6	44,3	49,8	68,5	42,8	53,2	43,2	60,2	53,3± 9,6
Apr-06	45,9	64,5	42,6	44,2	62,5	41,2	46,1	41,9	57,1	49,6± 9,2

Difference	10,9	27,4	17,5	16,1	28,4	18,6	13,7	18,6	27,2	19,8± 6,4
Loss (%)	19,3	29,8	29,1	26,7	31,2	31,1	22,91	30,7	32,3	28,1± 4,4

S =Sample

For the Rosemary Honey, Serra ¹ obtained average values of 14.4±5.7 SN (Range: 24 - 6) which are equivalent to 105.9±42.1 U/kg, (Range: 176.3 U/kg y 44.1 U/kg. These values are very different and denote the influence in this parameter of other flora that accompanies every monofloral honey.

After 10 months at ambient temperature, the rosemary honey lost between 19.28% and 32.27% of the initial values of α -glucosidase, with an average of 28.1%±4.4%.

4. CONCLUSION

As we observed, the values of the content of invertase in the two varieties studied conform to the regulations of the Granada Honey Protected Denomination of Origin Regulatory Board (Official State Bulletin number 301)¹³. And this is maintained at adequate levels during at least one year, making it necessary to make a fresh study of this parameter after a year. These are the first data of evolution of α -glucosidase in Granada's D.O.P. honey.

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