THE EFFECT OF HEATING ON HONEY HMF AND INVERTASE

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Abstract
Five honeys of diferent botanical origin were heated at 35,45,55,65 and 75oC for 24h. The decrement of invertase and the increase of HMF have been determined with harmonized methods. The role of invertase and HMF as criteria of heating indication is discussed. HMF can be used for the determination of overexposure to heat while invertase can detect gentle heating.

Key words*: honey /invertase/HMF/heating*

Introduction

To place honey in the market requires processing operations which includes the exposure to heat. Diastase activity and hydroxymethylfurfural (HMF) are considered as the main parameters for evaluating the freshness and the heat and storage history of honey. Many authors have expressed different opinions about the propriety of those parameters. WHITE (1992, 1994) in a series of articles criticizes severely the use of diastase content as quality criterion. He suggests that diastase content is not useful in evaluating honey quality and especially heating while HMF is more appropriate and can 'provide all the information needed to estimate the total heat exposure of any honey'. In a previous study WHITE (1964) demonstrated that invertase is preferable than diastase as it is more sensitive to heating. DUSTMANN (1993) maintained that 'invertase in combination with other analytical criteria can detect damage by heating or overstorage' and also 'HMF is rather inappropriate for the proof of heat damage, if taken into account as a sole criterion'. Invertase is responsible for the conversion of sucrose to fructose and glucose. It is produced from the hypopharyngeal glands of honeybee, added to the nectar by the bee (WHITE, 1975) and it is responsible for the ripening of nectar to honey. The amount of the enzyme depends on the age of the bee (BROUWERS 1982, BROUWERS 1983), the stage of the colony (HUANG et al.,1989), the nectar flow, the environmental conditions (WHITE, 1975) and the beekeeping practices (LAUDE et al.,1991).

WHITE et al. (1964) has studied the effect of storage and procession temperature on invertase, diastase and HMF. TAKENAKA and ECHIGO (1974) examined the decrease of invertase and diastase during storage and DUSTMANN et al. (1985) measured the accuracy of invertase methods. The invertase was determined by BOGDANOV et al. (1987) in Swiss and foreign honeys, ALDCORN et al. (1985) in Canadian honeys, LAUDE et al. (1991) in honeys from Philippines, HUIDOBRO et al. (1995) in Spanish honeys, KARABOURNIOTI and DRIMJIAS (1997) and TSIGOURI and PASSALOGLOU (2000) in Greek honeys and L. PERSANO ODDO et al. (1996,1999) in Italian honeys.

The aim of the present study is to observe the loss of invertase activity and the rise of HMF during heating at diferent temperatures and also to study the combination of those parameters as heating indicators. The selection of the samples of diferent botanical origins and starting values was on purpose in order to detect the effect of heating in samples with different initial values and resistance to heat.

Materials and methods

Five samples from different origin (pine, thymus, cotton, helianthus and orange) were divided in samples of 500 gr. The botanical origin was determined organolepticaly, by electrical conductivity and pollen analysis according to the of LOUVEAUX et al. (1978) method. One sample was kept unheated and the rest were placed in water bath for 24 h in 35, 45, 55, 65 and 75 oC. The samples were 2001 crop, except helianthus which was 2000 crop and had been stored for about a year and this explain the high HMF content at the unheated sample. At all 2001 samples, the time between extraction and experiment was 10 days to 3 months and they have been stored at room temperature. We analyzed the samples for HMF and invertase immediately after heating.

Invertase activity was determined according to the method of SIGNT-HALER (1977) which is based on the spectrophotometric measurement of decomposition of p-Nitrophenyl-a-D-glucopyrinoside(p-NPG) in p-nitrophenol at 400 nm. The results are expressed in units of the enzyme per kilogram. (U/Kg). HMF was measured according to the method of WHITE (1979) and was based on the determination of UV absorbance of HMF at 284 nm. The results are expressed in milligrams per kilogram (mg/Kg). The analysis was carried out using a Hitachi U-2001 UV-vis double-bean spectrophotometer.

Results

Table 1 shows the values of HMF and invertase at the five samples in different temperatures. As we expect there is an increase of HMF and a decrease of invertase after heating. We notice that the resistance to heat is different according to the botanical origin of the honey. The most resistant is the pine sample as well as the orange sample and followed by thymus, cotton and helianthus sample.

Table I

The effect of heating on invertase and HMF

	Pine		Orange		Helianthus		Cotton		Thymus	
Temp.	HMF	Invertase	HMF	Invertase	HMF	Invertase	HMF	Invertase	HMF	Invertase
Unheated	1.20	200.30	2.25	23.85	26.80	93.00	9.70	104.10	8.78	70.64
35	1.95	179.30	3.45	18.90	29.20	90.10	9.90	96.50	10.78	65.64
45	2.25	174.50	3.75	12.70	32.60	72.50	11.40	74.20	13.17	53.56
55	4.80	121.30	4.35	10.80	39.00	28.90	16.50	32.40	23.95	20.66
65	12.40	10.65	19.00	3.50	87.60	2.55	52.70	4.0	48.20	6.35
75	43.40	4.90	63.30	0	226.35	0	173.4	0	191.35	1.11

The decrease of invertase starts from the temperature of 35oC. From the results we have noticed that heating at 55oC for 24h period does not cause a significant increase of HMF. In fact, all the samples, including helianthus, were below the level of 40 mg/kg. The concentration of invertase at 55oC was decreased to less than the half of its initial value at pine, about half of its initial value at orange sample and about seventy percent at cotton, thymus and helianthus. At 65oC HMF at pine and orange sample is still low, while at the rest of the samples exceed the 40 mg/kg. The decrease of invertase is about ninety five percent at pine, helianthus, cotton and thymus and eighty five at orange honey. Despite the fact that orange sample show a lower level of initial value, it also shows highest resistance at the decomposition of the enzyme if we compare it with the other samples with higher values. At 75oC the enzyme was almost destroyed and HMF is extremely high except in pine honey which just exceeded 40 mg/kg.

Discussion

The decomposition of invertase is very fast and starts from 35o C, temperature which in many countries can be obtained during summer. The original value of invertase depends on the origin of honey which show a great variation. To use invertase as heating indicator presupposes the knowledge of the range of the enzyme according to honey origin and the original value of honey if we have mixtures. In addition, as demonstrated from PERSANO ODDO (1999), the difficulties of setting a general a limit for the enzyme are great as 'any limit could be unfairly severe for some honeys and too permissive for others'.

The HMF is the most important and reliable criterion to detect heating of honey as it has the advantage for not be present in fresh honey. As well as invertase, depends on the botanical origin of the honey and due to the variety of pH and acidity appear variations between blossom and honeydew honeys. Furthermore it allows in some honey sorts the overexposure to high temperatures as it can rise slowly. Exposure to rather low temperatures like 40-50oC is not easily detected.

Due to the fact that both invertase and HMF show limitations the combination of analytical criteria seams to be the safer way in order to detect the exposure to heat as invertase can detect gentle heating and HMF gives information about overtime heating.

REFERENCES

[1] Aldcorn, D L; Wandler, E; Sporns, P (1985) Diastase(a and ß-amylase) and a-glucosidase(sucrase) activity in western Canadian honeys. Canadian Institute of Food Science and Technology Journal 18(3): 268-270.
[2] Bogdanov, S; Rieder, K; Ruegg, M (1987) Neue Qualitastkriterien bei Honigntersuchungen. Apidologie 18(3): 267-278.
[3] Brouers EVM (1982)Measurement of hypopharygeal gland activity in the honeybee Journal of Apicultural Research 21(4): 193-198.
[4] Brouers EVM (1983)Activation of hypopharygeal glandsof honeybees in winter Journal of Apicultural Research 22(3): 137-141.
[5] Dustmann, JH; Praagh, JP; Bote, K (1985) Zur bestimmung von diastase, invertase und HMF in honig. Apidologie 16(1): 19-30.
[6] Dustmann JH(1993) Honey quality and its control. American Bee Journal 133(9): 648-651.
[7] Huang,ZY; Otis, GW; Teal,PEA (1989) Nature of broad signal activity the protein synthesis of hypopharygeal gland in honeybee Apis mellifera(Apidae:Hymenoptera) Apidologie 20, 455-464.
[8] Huidobro, JF; Santana, FJ; Sanchez, MP; Sancho, MT; Muniategui, S; Simal-Lozano J(1995) Diastase, invertase and ß-glucosidase activities in fresh honey from north-west Spain. Journal of Apicultural Research 34(1): 39-44.
[9] Karabournioti S; Drimjias N (1997) Some physicochemical characteristics of Greek monofloral honeys Apiacta XXXII 44-50.
[10] Laude, VT; Naegel, L; Horn, H (1991) Die physiko-chemischem Eigenschaften Philippinischer Honige Apidologie 22, 371-380.
[11] Louveaux, J; Maurizio, A; Vorwohl, G (1978) Methods of melissopalynology. Bee World 59(4): 139-157.
[12] Siegenthaler U (1977) Eine einfache und rasche Methode zur Bestimmung der a-Glucosidase(Sacharase) in Honig. Mitt Geb Lebensmittelunters Hyg 68, 251-258.
[13] Takenaka, T; Echigo, T (1974) Changes in enzyme activity during the storage of honey. Bulletin of the Faculty of Agriculture, Tamagawa University no 14: 19-25.
[14] Tsigouri A, Passaloglou M (2000) A scientific note on the charactreristics of thyme honey from the Greek island of Kithira. Apidologie 31, 457-458.
[15] Persano Oddo, L; Pulcini, P; Piazza, MG ((1996) Invertase content of Italian unifloral honeys. Apicoltore Moderno 87 (1) 25-29.
[16] Persano Oddo, L; Piazza, MG; Pulcini, P((1999) Invertase activity in honey. Apidologie 30: 57-65.
[17] White, J; Kushnir, I; Subers M H (1964) Effect of storage and processing Temperatures on honey quality. Food Technology 18(4): 153-156.
[18] White, JW (1975) Composition of honey, in Crane E. (Ed), Honey: A Comprehensive Survvey, Heinemann, London pp. 180-194.
[19] White, JW(1979) Spectrophotometric method for hydroxymethylfurfural in honey. J Assoc Off Anal Chem 62, 509.
[20] White, JW (1992)Quality evaluation of honey: Role of HMF and diastase assays in honey quality evaluation. American Bee Journal 132(11/12): 737-742, 792-794.
[21] White, JW (1994) The role of HMF and diastase assays in honey quality evaluation. Bee World 75(3): 104-117.