

**AN ATTEMPT TO ESTABLISH RELIABLE "BEST BEFORE" DATES FOR HONEYS
ORIGINATING IN BOTH CONTINENTAL AND OCEANIC CLIMATES**

Cavia, MM¹; Fernández-Muiño, MA¹; Alonso-Torre, SR¹;
Moreno, G¹; Mato, I²; Huidobro, JF²; and Sancho, MT^{1*}

¹ Universidad de Burgos. Departamento de Biotecnología y Ciencia de los Alimentos. Area de Nutricion y Bromatología. Facultad de Ciencias. Plaza de Misael Banuelos Garcia s/n. 09001 BURGOS (Castilla y Leon). SPAIN.

²Universidad de Santiago de Compostela Departamento de Química Analítica, Nutrición y Bromatología. Facultad de Farmacia. SANTIAGO DE COMPOSTELA (Galicia) SPAIN.

*Presenting Author and author for correspondence. M.T. Sancho. mtsancho@ubu.es. Facultad de Ciencias. Universidad de Burgos. Plaza de Misael Bañuelos s/n, E-09001 Burgos (Spain). Phone: 34-947-258-813. FAX: 34-947-258-831.

ABSTRACT

Honey is increasingly appreciated as a sweetening by European consumers. Nowadays, it is of particular importance to know how long honeys will last before they lose their quality. The purpose of this work was to research into "best before dates once opened" for honeys originating in continental ("C" samples) and oceanic ("O" samples) climates. Evolution, throughout thirty months, of several quality control parameters was determined. The study was carried out on 60 high quality unheated Spanish honeys (35 "C" samples and 25 "O" samples). All honeys were kept at room temperature, as they are usually stored both at home and at the supermarket. All parameters were analysed each five months by applying either official methods or equivalent procedures. Results are discussed with regard to average values. Electrical conductivity was not modified. Moisture kept constant during twenty months in both "C" and "O" samples, and then it decreased. Fructose and glucose decreased whereas HMF increased in all samples, but more markedly after 20 months. pH of both "C" and "O" samples kept constant during 15 months and then steadily decreased. In general, free acid showed a similar behaviour in both "C" and "O" samples with similar increases and decreases until 20 months and then, this parameter increased in all samples. Diastase activity decreased steadily in all samples. Acid phosphatase decreased steadily until 15 months in "C" samples and until 20 months in "O" samples then, it increased and decreased again in both group of samples, showing unpredictable evolutions. Glycerol contents steadily increased after 20 months. In conclusion, changes of honey composition with regard to several chemical parameters are marked after 20 months. Thus, 20 months could be proposed as a "best before dates once opened" for honeys originating in both continental and oceanic climates.

Key words: Honey, best before dates once opened, electrical conductivity, moisture, monosaccharides, acidity, hydroxymethylfurfural, diastase activity, acid phosphatase, glycerol.

INTRODUCTION

According to the European Council directive 2001/110/EC, the commercial quality of honeys is defined by sugar content, moisture content, water-insoluble content, electrical conductivity, free acid, diastase activity, and hydroxymethylfurfural (HMF) content (OJEC, 2002).

For any food, the original quality lasts until the “best before” date or the “use-by date”. “Best before dates” are related to the period of time during which foods remain fresh and keep their nutritional value, if they are properly stored. “Use-by dates” are related to health and safety, and are used for perishable foods.

The quality of different types of honeys has been widely studied. Up to 1980, Crane (1975) and White (1978), among other authors, compiled relevant literature about honey. In 2004, the journal *Apidologie* published a special issue on European unifloral honeys with eight articles, each of which dealt with different honey topics and commented recent literature references on honey research.

The purpose of this work has been to try to establish a “best before once opened” for honeys stored at room temperature, after studying the evolution of several quality control parameters potentially modifiables by aging. As far as we know, this study has not been carried out in honeys, and is interesting not only for scientists and beekeepers, but for the consumers who normally store the honeys at home (after using them for the first time) during long periods of time.

MATERIALS AND METHODS

The study was carried out on 60 high quality unheated Spaniard honeys, 35 of which, coming from an area with typical Continental climate ("C" samples) and 25 of which, originating in an area with typical Oceanic climate ("O" samples). All honeys were kept at room temperature, as they are usually stored both at home and at the supermarket.

The parameters analyzed were electrical conductivity, moisture, fructose, glucose, HMF, pH, free acid, lactones, diastase activity, acid phosphatase activity and glycerol content. All parameters were analysed each five months at 5, 10, 15, 20, 25 and 30 months by applying either official methods (Bogdanov et al., 1997; AOAC, 2002) or other reliable procedures (Günther and Burckhart, 1967; Louveaux et al., 1978; Huidobro and Simal, 1984; Sancho et al., 1991a; Terradillos et al., 1994, and Fernández-Muiño et al, 1996). Possible relationships between different parameters were researched (Statgraphics plus 4.0, 1997).

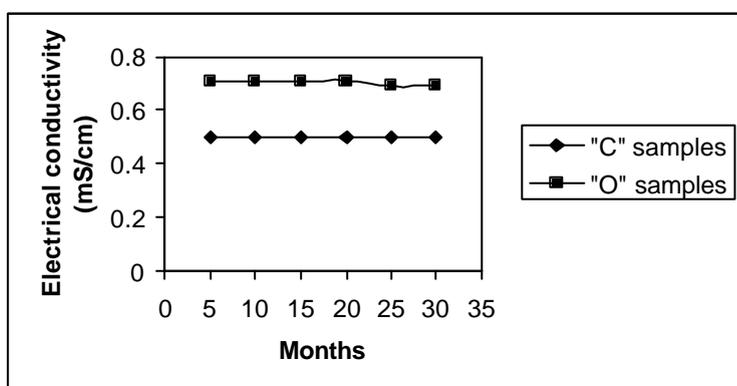
According to the melissopalynology, 14% of "C" honeys and 16% of "O" honeys were unifloral. The main taxa found in "C" samples were Ericaceae, *Lavandula* sp., *Helianthus annuus*, Leguminosae type *Trifolium* sp. and *Thymus* sp. In "O" samples the most important taxa found were *Castanea sativa*, *Eucalyptus* sp., Cruciferae and Leguminosae type *Genista* sp.

RESULTS AND DISCUSSION

In general, we did not observe different evolution patterns in honeys from different botanical sources, because we did not have a significant number of unifloral honeys from the same source. We corroborated that the evolution patterns of averages were similar to those of the vast majority of the samples.

Electrical conductivity is related to all types of ash of honey and their alkalinity (Accorti et al., 1987; Sancho et al., 1991b and Sancho et al., 1992), as well as to the botanical origin of this food (Crane, 1975). As expected, electrical conductivity kept constant throughout the study (Figure 1).

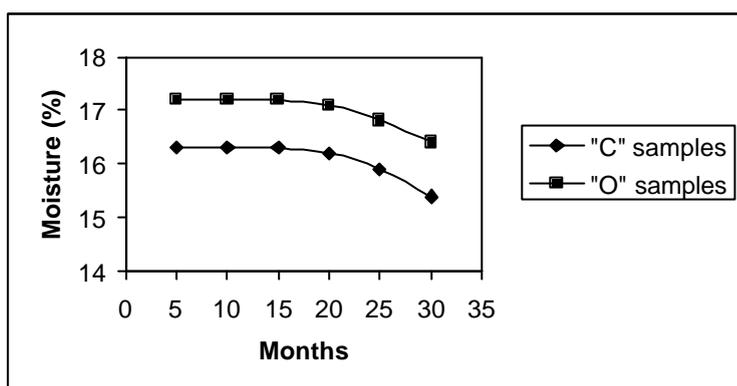
Figure 1. Evolution of mean values of electrical conductivity (mS/cm)



Moisture and sugar contents are related to the ripening of honey, and they influence viscosity, density, organoleptic properties, granulation and fermentation (Crane, 1975; White, 1978). A direct relationship was found between moisture and water activity of honeys (Cavia et al., 2004). In our study, water content of all honeys kept constant up to 20 months. Then, it decreased probably due to evaporations (Figure 2). We found moisture differences between "C" honeys and "O" samples depending on both climate

and flora. Fructose decreased continuously after 15 months in “C” honeys, and after 20 months in “O” honeys. No relation was found between initial moisture percentage and fructose decrease. Glucose decreased in all samples although in “C” honeys it kept more or less constant up to 20 months (Cavia, 2002; Cavia et al., 2002).

Figure 2. Evolution of moisture averages (%)



Free acid is considered a parameter of honey spoilage. pH and acidity types are influenced by the botanical origin of honey and are related to honey flavour and indirectly, to honey fermentation (Crane, 1975; White, 1978). In all honeys, we observed that pH steadily decreased after 15 months. Up to 20 months, both free acid and lactones continuously changed, due to the balance acids-lactones. Nevertheless, free acid increased whereas lactones decreased continuously after 20 months (Cavia et al., 2005a and b). A relationship between pH and electrical conductivity was found. In “C” honeys, the lower the initial pH was, the greater the decrease of fructose was. In “O” honeys, the lower the initial pH was, the more marked the decrease of fructose and glucose was. We also observed that between 20 and 25 months, the higher the moisture, the greater the increase of free acid. After 25 months there was a significant increase of the number of honeys with high values of free acid. With respect to lactones,

the higher the pH was, the lower the lactone acidity was, and the lesser the lactones changed with time.

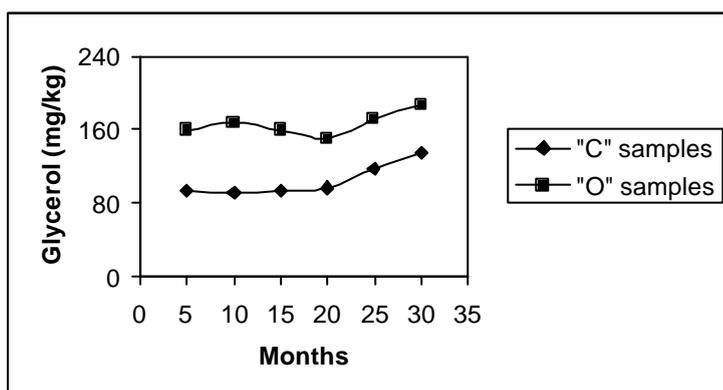
HMF is an aldehyde formed during acid-catalysed dehydration of hexoses. HMF is a parameter related to honey spoilage (Crane, 1975; White, 1978; Anam and Dart, 1995). Its formation depends on temperature of processing and storage, high moisture and acidity, metals (Mg, Mn, Fe, and Zn), aminoacids (alanine, aspartic acid, and others), and sugars (fructose and glucose), among other factors. HMF is a quality parameter of honey because it modifies colour and flavour of this food. In the samples analyzed, HMF continuously increased but much more markedly, after 20 months (Cavia et al., 2005c). We did not observe that the lower the pH was, the greater the increase of HMF was. However, honeys with higher initial percentages of both moisture and fructose and lower initial percentages of glucose underwent greater increases of HMF.

Diastase activity is related to honey quality and aging. Diastase activity depends on botanical origin of honey, as well as on the temperature of processing and storage (Crane, 1975; White, 1978). Honey acid phosphatase is a less studied enzyme, whose values depend on botanical origin and are related to honey's spoilage by fermentation (Giri, 1938; Ivanov, 1978). In our samples, diastase activity decreased, but we observed two different slopes of decrease, one up to 20 months, and another after 20 months. In "C" honeys, acid phosphatase activity decreased up to 15 months, and increased from 15 to 20 months. In "O" honeys, acid phosphatase activity decreased up to 20 months, and increased from 20 to 25 months. Then, this enzyme decreased continuously (Alonso-Torre et al., 2005). We corroborated that the initial activity of such enzymes as diastase and acid phosphatase depended on honeys' botanical origin. Statistical analysis made it clear that initial enzymatic activities showed a significant influence on

their percentage of variation during storage. In general, the lower the pH was, the lower the diastase activity was. We also observed that the higher the pH was, the lesser the decrease of acid phosphatase activity was. In "C" honeys, pH and acid phosphatase activity values were correlated throughout the study.

Glycerol is a product of honey fermentation whose initial values depend on honey's botanical origin (Laub and Marx, 1987). In all "C" and "O" samples, glycerol contents kept constant up to 20 months. Then, glycerol continuously increased (Figure 3).

Figure 3. Evolution of glycerol (mg/kg) averages



In conclusion, the studied honeys kept their original quality during 20 months. After 20 months, we observed a continuous increase of free acid, HMF and glycerol contents and a continuous decrease of moisture and lactones. Thus, on the basis of the parameters analyzed, 20 months could be proposed for honeys coming from Continental and Oceanic Climates as "best before date once opened".

ACKNOWLEDGEMENTS

The authors are grateful to the Spanish JUNTA DE CASTILLA Y LEÓN and INIA (Ministerio de Ciencia y Tecnología) for supporting this work under projects No. BU17/99, and API98-007. Thanks are also due to all the beekeepers who have provided the honey samples for this study. The authors also acknowledge Apimondia with thanks for choosing this work for oral presentation in Dublin on 23rd August 2005.

LITERATURE REFERENCES

Accorti, M.; Piazza, M.G. and Persano Oddo, L. (1987). La conductivité électrique et le contenu en cendre du miel. *Apiacta* 22, 19-20.

Alonso-Torre, SR., Cavia, MM., Fernández-Muiño, MA., Moreno, G., Huidobro, JF. and Sancho, MT. (2005). Evolution of Acid Phosphatase Activity of Honeys from different climates. *Food Chem.*, Accepted for publication.

Anam, O.O. and Dart, R.K. (1995). Influence of metal ions on hydroxymethylfurfural formation in honey. *Anal. Proc. Inc. Anal. Comm.*, 32(12): 515-517.

AOAC (2002) Official Methods of Analysis of AOAC International. (17th edition). Arlington, Virginia. USA.

APIDOLOGIE (2004). Vol. 35. No. Suppl. 1. Special Issue: European unifloral honeys.

Bogdanov S., Martin P., Lüllmann C. (1997) Harmonised methods of the European Honey Commission. *Apidologie*, extra issue, 1-59.

Cavia, M.M. (2002). Estudio del envejecimiento de mieles de Burgos y Galicia: Influencia de la granulación inducida. Ph.D. Thesis. Supervisors: M.T. Sancho, M.A. Fernández-Muiño and J.F. Huidobro. University of Burgos. Spain.

Cavia, M.M.; Fernández-Muiño, M.A.; Gómez-Alonso, E.; Montes-Pérez, M.J.; Huidobro, J.F. and Sancho, M.T. (2002). Evolution of fructose and glucose in honey over one year: influence of induced granulation. *Food Chem.* 78, 157-161.

Cavia, M.M, Fernández-Muiño, M.A., Huidobro, J.F. and Sancho, M.T. (2004). Correlation between Moisture and Water Activity of Honeys Harvested in Different Years. *J. Food Sci.* 69(5), 368-70.

Cavia, M.M.; Fernández-Muiño, M.A.; Alonso-Torre, S.R.; Huidobro, J.F. and Sancho, M.T. (2005a). Evolution of acidity of honeys from Continental climates: Influence of induced granulation. *Food Chemistry*. Submitted.

Cavia, M.M.; Fernández-Muiño, M.A.; Huidobro, J.F. and Sancho, M.T. (2005b). Evolution of acidity of honeys from Oceanic climate: Influence of induced granulation. In preparation.

Cavia, M.M.; Álvarez, C.; Martínez, C.; Huidobro, J.F.; Fernández-Muiño, M.A. and Sancho, M.T. (2005c). Evolution of hydroxymethylfurfural content of honeys from different climates: Influence of induced granulation. In preparation.

Crane, E. (1975). Honey: A comprehensive survey. Heinemann, London.

Fernández-Muiño, M.A.; Sancho, M.T.; Muniategui, S.; Huidobro, J.F.; Sánchez, M.P. and Simal-Lozano, J. (1996). Direct enzymatic analysis of glycerol in honey: a simplified method. *J. Sci. Food Agric.* 71, 141-144.

Giri, KV. (1938). The chemical composition and enzyme content of indian honey. *Madras Agric J.*, XXVI (2), 68-72.

Günther, F. and Burckhart, O. (1967). Bestimmung der Sauren Gesamtphosphatase in Honig. *Deut. Lebens.-Rundsch.* 63(2), 41-44.

Huidobro, J.F. and Simal, J. (1984). Determinación de los azúcares de la miel. *An. Bromatol.* 36(2), 247-264.

Ivanov, T. (1978). Study of invertase, acid and alkaline phosphatase and esterase activities in bee honey. *Zhivonovud Nauk.*, 15 (3), 103-112.

Laub, E. and Marx, R. (1987). Glycerin, a natural ingredient of honey. *Lebensmittelchem. Gerichtl. Chem.*, 41, 110.

Louveaux J., Maurizio A., Vorwohl, G. (1978) International Commission for Bee Botany of IUBS. *Methods of Melissopalynology.* *Bee World* 59, 139-157.

OJEC-Official Journal of the European Communities. (2002) Council Directive 2001/110/EC of 20 December 2001 relating to honey.

Sancho, M.T.; Muniategui, S.; Huidobro, J.F. and Simal, J. (1991a). Correlation between the electrical conductivity of Honey in humid matter and dry matter. *Apidologie* 22, 221-227.

Sancho, M.T.; Muniategui, S.; Sánchez, M.P.; Huidobro, J.F. and Simal, J. (1991b). Relationships between electrical conductivity and total and sulphated ash contents in Basque honeys. *Apidologie* 22, 487-494.

Sancho, M.T.; Muniategui, S.; Sánchez, M.P.; Huidobro, J.F. and Simal-Lozano, J. (1992). Evaluating soluble and insoluble ash, alkalinity of soluble and insoluble ash and total alkalinity of ash in honey using electrical conductivity measurements at 20 °C. *Apidologie* 23, 291-297.

Statgraphics for Windows 4.0 plus (1997) Manugistics Inc. Rockville, Maryland. USA.

Terradillos, L.A.; Muniategui, S.; Sancho, M.T.; Huidobro, J.F. and Simal-Lozano, J. (1994). An alternative method for analysis of Honey sediment. *Bee Science* 3(2), 86-93.

White JWJr. (1978) Honey. In *Advances in Food Research*. Ed. Board Academic Press, New York, San Francisco, London, vol. 24, pp. 288-354.