Riscos e Alimentos

Portuguese Food Safety Authority Scientific Magazine

Honey

Bee health: How EFSA is helping to protect our pollinators

Honey quality

Infant botulism in Portugal

ASAE Autoridade de Segurança Alimentar e Económica

Nr. 6 - december 2013

CONTENTS

Editorial

Pedro Portugal Gaspar
General Inspector of ASAE

Into ASAE communication policy several instruments converge, some of them are more informative, namely the website or ASAENews, while others have a more scientific content and consequently increased programmatic characteristics, as is indeed the case of the publication "Riscos e Alimentos".

Both these communication lines are not only necessary but complementary, thus corresponding to a well-defined option and strategy by ASAE, because this authority must pursue a course of inspection and supervision properly supported in a scientific knowledge, which obviously must be divulged and enhanced.

This way, in this extensive mission, specifically concerning the risk assessment and communication in the food chain, ASAE biannually publishes the scientific newsletter "Riscos e Alimentos". This publication is prepared by the Food Risk and Laboratories Department, with the collaboration of scientific partners, specially the members of the Scientific Council and the Thematic Panels, as well as Universities and National and European Authorities, which contribute to the development and diffusion of ASAE's work of in the area of risk assessment and communication, always in close cooperation with EFSA, since ASAE is the national Focal Point.

So, in this first publication of "Riscos e Alimentos" under my commission, I must sign the respective editorial precisely to underline the importance that the preventive activity which ASAE now assumes in this strategy which trace the new cycle management. Thus, it is necessary to intensify the importance of laboratory activity and risk assessment and communication, key activities of ASAE, as well as the importance of an effective institutional cooperation which provide increased national scientific and technical contribution, mainly on sharing information.

New Scientific of ASAE

Jorge Reis
General Subinspector of ASAE

ASAE's Scientific Council is a specialized consultive organization to give advice and to monitor the food risks issues, reporting to of the manager of the food risks department. The Scientific Council offers its expertise to advice on scientific matters, on technological development and research projects. It has full technical and scientific autonomy to this purpose.

This Scientific Council is composed by three to six experts with recognized scientific merit appointed by the member of government responsible for the economy department, under proposal of the General Inspector of ASAE and selected amongst universities professors and researchers of several specialties, including Risk Communication.

Since its nomination in 2006, the first Scientific Council has provided scientific and technical support to ASAE's scientific and operational activity, especially when the services require additional scientific support, due to the highly technicality and complexity of several subjects. The support of the Scientific Council is also important with the risk criteria associated to foodstuff and activities, as well as some situations involving potential hazards in the food chain unknown or not contemplated in the legislation.

This support has been embodied in particular by the provision of scientific opinions, monitoring of scientific and technical process in food safety, evaluating risks in food chain, proposing several studies, with the presence of its members in discussions and seminars, and the appointment and activation of Thematic Panels whenever it is necessary given the specificity of the subjects at issue.

With the publication of the new Organic Law of the ASAE there was the necessity to nominate a new Scientific Council, which was formalized by publication of Order number 15074/2013, from Mr. Secretary of State Assistant of Economy. The acknowledged scientific merit of these personalities - some of them passed from outgoing council - as well as the academic institutions represented by these personalities, undoubtedly give us an expectation of assured continuity of the work previously carried out in this area, with the responsibility and the capacity of intervention that we want to see increased in this new management cycle in ASAE, which will give a special attention to activities related to riskassessment and communication in the food chain.

At last, I want to publicly thank to Professor Artur Manuel Soares da Silva, and posthumously to Professor Ana Cristina Gaspar Nunes Lobo Vilela, the excellent work and prestigious collaboration that always gave in performance of their duties in the outgoing Scientific Council.

ASAE
Autoridade de Segurança Alimentar e Económica

Bee health: how EFSA is helping to protect our pollinators

Simon Terry
European Food Safety Authority (EFSA)

Beekeeping is an ancient tradition, and honey bees have been kept in Europe for several millennia. Bees are critically important in the environment, sustaining biodiversity by providing essential pollination for a wide range of crops and wild plants. They contribute to human wealth and wellbeing directly through the production of honey and other food and feed supplies such as: pollen, wax for food processing, propolis in food technology, and royal jelly as a dietary supplement and ingredient in food.

The Food and Agriculture Organization of the United Nations estimates that of the 100 crop species that provide 90% of food worldwide, 71 are pollinated by bees. The majority of crops grown in the European Union depend on insect pollination. Beyond the essential value of pollination to maintaining biodiversity, the global annual monetary value of pollination has been estimated at hundreds of billions of euros.

Because of the important ecological and economic value of bees, it is vital that we monitor and maintain healthy bee stocks, not just locally or nationally, but globally.

Bees in decline

Over the past 10 to 15 years, beekeepers have been reporting unusual weakening of bee numbers and colony losses, particularly in Western European countries including France, Belgium, Switzerland, Germany, the UK, the Netherlands, Italy and Spain. In North America, colony losses observed since 2005 have left the region with fewer kept bees than at any time in the past 50 years. American scientists have coined the term Colony Collapse Disorder (CCD) to describe this phenomenon. CCD is often characterised by the rapid loss from a colony of its adult worker bee population.

No single cause of declining bee numbers has been identified. However, several possible contributing factors have been suggested, acting in combination or separately. These include the effects of intensive agriculture and pesticide use, starvation and poor bee nutrition, viruses, attacks by pathogens and invasive species – such as the Varroa mite (*Varroa destructor*), the Asian hornet (*Vespa velutina*), the small hive beetle *Aethina tumida* and the bee mite *Tropilaelaps* – genetically modified plants, and environmental changes (e.g. habitat fragmentation and loss).

EFSA's role

The European Food Safety Authority (EFSA) has an important role to play in ensuring that healthy bee stocks are maintained in Europe, given its mandate to improve EU food safety and animal health and to ensure a high level of consumer protection. A number of the Authority's scientific experts contribute to this work, principally in the areas of pesticides, animal health and welfare and plant health, genetically modified organisms (GMOs), data collection and scientific assessment.

Central to this work are the assessments EFSA carries out of the environmental safety of pesticides and GMOs that manufacturers would like to place on the EU market, and the advice provided by EFSA's plant health experts on the risks posed by organisms that can cause harm to plants, plant products or plant biodiversity in the European Community.

EFSA's pesticides experts have carried out a number of high-profile risk assessments that have helped to inform EU policy on bee health. In 2012 they assessed the potential effects on bees of thiamethoxam, imidacloprid and clothianidin, which are members of the neonicotinoid group of pesticides. The assessments, published earlier this year, paid particular attention to acute and chronic effects on bee colony survival and development, taking into account the effects on bee larvae as well as bee behaviour. The European Commission subsequently introduced restrictions on the use of these pesticides in Europe.

In May 2013 EFSA performed a risk assessment of the insecticide fipronil, paying particular regard to the possible acute, chronic and sub-lethal effects on bees. The Commission reacted by imposing restrictions on the use of fipronil.

Another significant piece of work published this year - this time by EFSA's animal health and welfare experts – was an assessment of the risk of introduction and spread in the EU of the small hive beetle (*Aethina tumida*) and the *Tropilaelaps* bee mite through the import of live bees and bee products, and of products such as fruit and vegetables.

More broadly, EFSA has an internal task force which is carrying out an overview of the state of risk assessment in the area of bees. The task force published a report in November 2012 giving an overview of EFSA's current activities and making recommendations on how this work should be continued. It will publish a second report in early 2014 looking at how EFSA can best collaborate with other organisations to advance knowledge in this area.

The formation of the task force has its roots in 2009, when EFSA launched a project to assess bee surveillance systems in the EU, and to collate and analyse data and publications related to honey bee colony mortality across Europe. The subsequent report *Bee Mortality and Bee Surveillance in Europe* made a number of recommendations to improve surveillance as well as identifying consensus across the EU on the multifactorial origins of the decline in bee numbers. It also helped to shape the European Commission's strategy for tackling the decline in bee numbers across Europe, which was clarified in a key communication on honey bee health published in 2010. In May 2012, the Commission allocated €3.3 million to support 17 Member States carrying out surveillance studies related to losses of honey bee colonies.

efsa
European Food Safety Authority
Committed *since 2002* to ensuring that Europe's food is safe

Bee Health Program

The importance of official control in the production and marketing of honey

Sofia Quintans
squintans@dgav.pt

Susana G. Freitas
susana.freitas@dgav.pt

Directorate General of Food and Veterinary (Directorate for Animal Protection – Unit for Epidemiology and Animal Health)
Largo da Academia Nacional de Belas Artes, nº 2, 1249-105 Lisboa;

Introduction

The population of bees (Apis mellifera) of the EU plays an important role, both in pollination and the production of honey and other bee products (wax, royal jelly, etc...). This is the reason why the EU has set certain standard rules to protect and maintain the health of bees.

It is important to protect bees' health in a proactive way, particularly taking into account the specificities of apiculture and the various stakeholders involved. Beekeeping/ apiculture is a widely developed activity in the EU, both professionally (beekeepers with over 150 hives), and as a hobby. The pollination of flowering plants, wild and cultivated, is essential in order that life continues on the planet. Honey is the most popular of beekeeping products. Traditionally, in most societies, the production of honey can create means of subsistence and development in various sectors (Bradbear, N. 2005).

According to EUROSTAT, honey production in the EU was 217 366 tons in 2011. EU production increased slightly in the last ten years (+ 6% since 2010.) The three major honey producers in the EU are Spain, Germany and Romania, with a production of, respectively, 34 000, 25 831 and 24 127 tons in 2011. Hungary (19 800 tons), France (16 000 tons), Greece (14 300 tons) and Poland (13 369 tons) are also important producers.

According to FAO, the world production of honey in 2011 was 1.636 million tons. In the last ten years the production has been increasing slowly but steadily, except for the years 2007 and 2009. China is the largest producer of honey, with 446 000 tons, representing 27.3% of the worldwide total, followed by the EU, with 217 000 tons. (13,3 %). The other main producers are Turkey, Ukraine and the United States. The EU and the U.S. are the two leading importers of honey.

In recent years, there has been an increase in bee mortality, both in the EU and elsewhere, which raised serious concerns around the world, but scientific studies have failed to determine the exact cause or severity of this phenomenon.

However, bees' health is connected to many factures of several natures (bacterial, viral, parasitic, etc...); availability of appropriate treatments; invasive species and environmental changes. Other factors to consider include the use of pesticides in agriculture.

Apiculture health – Official Control

The Directorate General of Food and Veterinary (DGAV) is the competent authority at central level responsible for the preparation, coordination and monitoring of the apiculture health program, 2013 (prepared in accordance with Decree-Law nr. 2003/2005 of 25 November) that aims at the establishment of animal health measures for the prevention and control of bee diseases in the national territory (Table 1), as well as the requirements to be satisfied by the controlled zones.

http://www.dgv.min-agricultura.pt/portal/page/portal/DGV.

It is the responsibility of the five Directorates of Food and Veterinary of DGAV Regions (Norte, Centro, Lisboa e Vale do Tejo, Alentejo e Algarve), and of the Regional Directorate and Rural Development of Açores and of the Regional Veterinary Directorate of Madeira, the control e execution of the different actions in their respective areas of influence.

Table I - Compulsorily notifiable diseases

American Foulbrood.
European Foulbrood.
Acarapisosis.
Varroosis.
Aethinose /*Aethina tumida.*
Tropilaelaps /*Tropilaelaps* sp.
Ascosferiosis (only in controlled areas).
Nosemosis (only in controlled areas).

The European Commission has proposed to every Member State (Execution Decision of the Commission of 17 October, 2013) the presentation of a pilot surveillance program, which Portugal joined to, in order to assess the causes of colony losses of bees in Europe, through strategies defined and concerted by the participating Member States, and this will enable to elaborate recommendations and proposals to delineate and improve surveillance systems in Europe.

In 2006, a National Epidemiological Screening for bee diseases was conducted, in a partnership between the Faculty of Veterinary Medicine of Lisbon, UTL, the National Veterinary Research Laboratory-INRB, the National Federation of Beekeepers of Portugal, Beekeepers organizations and the Directorate General of Food and Veterinary (DGAV), which is available on the website of the latter referred (DGAV), having been confirmed that the following bees' diseases are present in Portugal, in endemic form: Varroosis (Pic. 1) , American foulbrood (Pic.1), Acarapisose, Ascosferiose and Nosemosis.

Pic.: 1

Varrose-Foto: Anses ***Varrose-Foto: Anses*** ***Loque americana***

The positive laboratory results in recent years (analysis performed by individual beekeepers by beekeepers or the DGAV organizations) show an apparent increase of varroosis in recent years mainly due to the substantial increase of analyzes made by the sector and particularly by managing entities of controlled areas. That increased number of analyzes is due to the joint work of the state(DGAV / INIAV / LNIV) and the sector towards the awareness of beekeepers to the importance of laboratory tests for a correct diagnosis of diseases in apiaries and, therefore, for the accomplishment of the appropriate treatment and improvement of health conditions of the apiaries.

The control plan against varroosis was prepared within the framework of the National Apicultural Program 2011-2013, available on the website of the Bureau of Planning and Policy (http://www.gpp.pt/ma/apicultura/), approved by the European Commission in July 2010, with the view to forming a support tool for beekeepers and their organizations in the fight against varroosis in the national territory. Thus, the structure of this plan has taken into account the methodology proposed by DGAV for the National Apicultural Program for 2011-2013, under Regulation (EC) nr. 797/2004 of the Council, of 26 April, concerning the improvement actions of the production conditions and marketing of apiculture products.

As it is not possible to completely eradicate varroosis, treatment of the hives with approved methods and products is the only means to avoid the consequences of the disease.

The legislation in force also provides for the zoosanitary certification and some requirements for the movement of bees between Member States. These requirements are intended to prevent and control some bee diseases, namely the American and European foulbrood, the small hive beetle and *Tropilaelaps* mites, which can be spread through the movement of bees. The small hive beetle (*Aethina tumida*) and the *Tropilaelaps* mites are exotic to the EU, so their notification is mandatory so that Member States can take immediate action in case of an outbreak.

There are animal health requirements for imports from third countries of live bees and bumble bees (*Bombus spp*) specimens, so as to avoid bringing exotic bee diseases into the EU. These requirements are applied since 2005. Compliance with these health requirements is controlled upon the entry into EU veterinary border inspection posts, where identity and physical documentary controls are executed by official veterinarians.

The improvement of knowledge on bee health will be the best contribution to a safer food.

The specific requirements that the **Operators of the Honey sector and other Bee Products** must respect, are in general terms, without prejudice to the provisions in other specific legal diplomas, defined in Decree-Law nr. 1/2007 02 January. In Portugal, according to the Regulatory Decree nr. º 11/2007 of 27 February and with the Administrative Rule nr. 219-F/2007 February 28, it is the responsibility of DGAV, through the Directorate for Food Safety (DSSA), to coordinate the official health-hygiene control and the health inspection of fresh animal products, aiming at the safeguard of animal origin foodstuffs, animal health and genuineness of meats and animal origin products.

It is the responsibility of the Regional Directorates for Food and Veterinary to ensure the implementation of actions and execution of services, as defined by the DGAV central services.

Conclusion

The national apiculture programs aim to improve the production and marketing of the honey in the European Union.

The measures allow keeping the production of high quality honey in the EU, despite a difficult rising production price, threats to the survival of bees and the international competition context.

However, it is clear that the areas where it is necessary to make progress include, among others, enhanced biosafety and improved production practices from beekeepers, the development of new medicines, by the industrial sector, for bees, or the design of better programs of training for authorities and beekeepers.

Bibliography:

Bradbear, N. 2005 – Apicultura y los medios de vida sostenibles - "Dirección de Sistemas de Apoyo a la Agricultura Organización de las Naciones Unidas para la Agricultura y la Alimentación", Edi. FAO - Folleto sobre diversificación, Roma:

http://www.fao.org/home

Decisão de Execução da Comissão, de 17 de outubro de 2013.

Decreto-Lei nº 2003/2005, de 25 de novembro.

Decreto-Lei n.º 1/2007, de 02 janeiro.

Decreto-Regulamentar n.º 11/2007, de 27 de fevereiro.

Plano Sanitário Apícola 2013:

http://www.dgv.minagricultura.pt/portal/page/portal/DGV

Portaria n.º 219-F/2007, de 28 de fevereiro.

Regulamento (CE) n.º 797/2004 do Conselho, de 26 de abril.

Relatório da Comissão ao Parlamento Europeu, 2012 - Relatório da Comissão ao Parlamento Europeu e ao Conselho Sobre a Aplicação das Medidas Relativas ao Setor da Apicultura do Regulamento (CE) n.º 1234/2007 do Conselho:

http://ec.europa.eu/agriculture/evaluation/market-and-income-reports/index_en.htm.

Honey - The Use of Antibiotics and Pesticide

Elisa Carrilho
Food Risks Unit

According to the Decree-Law nº 214/2003 september 18, of 2003, the definition of honey is: "natural sweet substance produced by bees of the species *Apis mellifera* from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects staying on the living parts of plants that bees collect and transform by combining with specific substances that deposit, dehydrate, store and leave in honeycombs to ripen."

Beekeeping is a very significant activity in Europe. There are over 700,000 beekeepers in the EU and 97% of these are not professional.

According to Regional Direction of Agriculture of Entre Douro e Minho, on a 2006 publication, one of the weaknesses of the national beekeepers is the low level of training, being mostly a complementary activity.

There is a lack of central honey factories that allows improving hygiene conditions and food safety and self-organization of beekeepers.

One of the strong points of this paper refers, "flora and very good climatic conditions for the honey production of good quality and the production models installed at low cost, with an high productivity".

Last years there has been an increase in bee mortality, both within Europe and globally.

Most crops in the EU depend on pollination by insects and this is essential for biodiversity, and therefore it is of the most importance its monitoring to keep the health. It is evaluated that the annual value of this pollination in billions of euros.

There are several factors that influence the health of bees such as: bacteria, viruses, parasites; the existence or not of appropriate treatments; invasive species; changes in environment; pesticides in agriculture.

According to Council Directive 92/65/EEC, is expected zoosanitary accreditation and some requirements for the movement of bees between the member states.

The small hive beetle and mite *Tropilaelaps*, caused major damage to the beekeeping sector in countries that have been introduced, that´s why the import rules of the European Union establishes that "only queen bees and colonies of Bombus spp gender from plants biosecurity can be imported from third countries", according to the Commission Communication to the European Parliament and the Council, on the health of bees.

These requirements aim to prevent and control some diseases of bees, namely the American and European foulbrood (bacterial diseases).

The oxytetracycline was the first antibiotic to be used in the control of these diseases, and some others emerge as sulfonamides, streptomycin, or chloramphenicol. However these substances are not allowed at the national level. The decrease in the bee population in Europe and the rest of the world, it is a major concern because of the lack of appropriate medicines existing for the diseases in bees.

According to the beekeepers associations, there are insufficient authorized medicines to treat bee diseases.

This is because the market for veterinary medicines is small, and the return of companies investing is poorl.

However measures by the European Medicines Agency (EMEA) have been taken to promote innovation and development of new veterinary medicinal products for small and medium enterprises.

Varroasis is an external parasitic disease caused by the mite *Varroa destructor*, whose life and reproduction cycle runs in parallel with the bee, so there is widespread contamination which causes the disappearance of large numbers of colonies.

Was identified in Portugal in 1986, and has been responsible for the loss of many colonies, with the corresponding decrease of honey production.

According to the normative Order Nº. 27/2010, there are national candidates to support in the fight against varroa.

There are several factors that can contribute to bee diseases: intensive agriculture and the use of pesticides,_hunger and malnutrition of bees, viruses, the mite *Varroa destructor*, the Asian hornet (*Vespa velutina*),the bees beetle *Aethina tumida*,mite bee *Tropilaelaps,* genetically modified plants, and environmental changes.

EFSA is responsible for ensuring the number of bees in European because its main concern is to improve food safety animal health in UE to ensure a high level of safety for the consumer.

A large number of Scientific Panels and EFSA Units contribute to this work, mainly in the area of pesticides, animal health and welfare, plant health, genetically modified organisms (GMO), data collection and scientific evaluation.

Panel on Plant Protection Products and Their Residues (PPR), provides independent scientific advice on risk assessment of plant protection products and their residues.

This includes the risk of operators, workers, resident and consumers, as well as the environment, and wildlife.

The Plant Health Panel (PLH Panel) provides scientific advice on the risks posed by organisms that can cause damage to plants, to the products of plants, or to the biodiversity in the European Community.

EFSA's work in the area of plant health is particularly relevant to bee health, mainly concerning some pests that are a threat to bees, and they may be carried by them or live on the plants.

The Unit Diet and Monitoring Chemistry publishes an annual report, summarizing data on the presence of residues of veterinary drugs, and other substances in live animals and animal products (such as honey) in the European Union.

The last report in 2010, shows that only 0,33% of 418 081 samples were non-compliant, a value similar to that recorded in 2009 (0.32%).

Bees are covered by the Animal Health Strategy for the European Union 2007-2013 –"Better safe than sorry", followed, in 2008, by an Action Plan with specific actions on the certification of animal health and requirements for the movement of bees between member states (Directive 92/65/CEE).These requirements are intended to prevent and control bee diseases and pests.

In 2009, EFSA has introduced a project to evaluate the surveillance system of the bees in the EU, and to collate and analyze data and publications related to the mortality of bee colonies in Europe.

From this draft report was prepared "Mortality of bees and surveillance in Europe" where recommendations were made to improve surveillance, and to identify consensus throughout Europe of multifactorial origin of the declining of bees number.

The Commission in a Communication on bee health and honey published in 2010, helped to devise the strategy to combat the decline of bee number in Europe.

In 2012, the Emerging Risks Unit of EFSA participated in a working group created by ANSES (french authority) to review a scientific opinion on the combined impact of pathogens of bees with low doses of pesticides, the mortality of honey bees and to ensure closer scientific collaboration between EFSA and ANSES on the risk assessment of bees.

The working group concluded that more research was needed on the toxicokinetic characteristics of the chemical substances bees contact with the environment. And that new ways of assessing the potential risk to bees of plant protection products need to include the exposure of bees to small and repeated doses of pesticides.

In February 2012 the Office of Pesticides reviewed the risk of active substance in bees tiametoxane, as requested by the European Commission.

This substance belongs to the group of insecticides (neonicotinoids), which some studies suggest may be a contributing factor to the loss of bee colonies.

The use of neonicotinoids is restricted in Germany, Italy, France and Slovenia.

In January 2013 experts from the Animal Health and Welfare Panel of EFSA published a scientific opinion on the risk of introduction and spread in the EU, of the small hive beetle (*Aethina tumida*)_and *Tropilaelaps* mite through imports from third countries of live bees and bee products, and products like fruit and vegetables.

EFSA continued his work in this area carrying out a risk assessment of the potential effects about bees tiametoxane, imidacloprid and clothianidin.

The reviews published in January 2013 had a particular focus on acute and chronic effects on the survival and development of the colonies, taking into account effects on bee larvae and behavior of the bees.

Following the recommendations of the Working Group of EFSA / Anses, May 2013, EFSA did a risk assessment of the insecticide fipronil, giving particular attention to the possible acute effects chronic and sub-lethal on bees.

Later, that month more than 100 experts attended in "Scientific Authority's Coloquium" on global approaches to risk assessment of multiple stressors on the bees.

In July 2013 EFSA published a guidance document on risk assessment of pesticides in honey bees, solitary bees and drones.

To assist risk managers in making their decisions, the Authority in May 2012, had created a task force to compile the review of the work undertaken by EFSA, as well as current activities outside of EFSA in the bees area.

The task force, published a report in November 2012, giving an overview of the current activities of the EFSA and make recommendations on how this work should continue.

Panel PPR is constantly reviewing the guidance document to assess the risk of pesticides related to terrestrial and aquatic ecotoxicology.

One of the mentioned subject in this document will be the effect on bees of the interaction between pesticides and other stressors.

<u>Residues of antibiotics and other pharmacologically active substances</u>

According to the Regulation No. 377/2010 on pharmacologically active substances, there are no maximum residue limits set for antibiotics in honey

because the European Union is not authorized to use antibiotics in beekeeping so there is a policy of "zero tolerance" for the presence of antibiotics in honey.

Since 1997 it is compulsory for residues of pharmacologically active substances

(for the EU and third countries) in accordance with Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products.

The Decree-Law Nº 148/99 of May 4, establishes rules for the collection of official samples using the monitoring of certain substances and residues thereof in live animals and animal products.

The “Direção Geral de Alimentação e Veterinária” is the organism responsible for design, coordination and implementation of control systems, namely the National Residue Control Plan (NRCP) which complies with the law and is mandatory in all Member States.

The Portuguese Food and Economic Safety Authority made the samples of honey at farm, until 2011.

The analyzes have been performed at the National Laboratory of Veterinary Research, from random sampling taking into account the annual production.

According to data from DGAV, PNCR, in 2011, there were no non-compliant results in the analysis of honey, the same has been happening in 2012 so far (October 2012), search for the following chemicals:

Subgroups		Analyzed samples		Non compliant samples
B1	Antibacterial substances including sulfonamides and quinolones	Honey factory/ apiary	22	0
B2c	Carbamates and pyrethroids	Honey factory/ apiary	24	0
B3a	Organochlorine	Honey factory/ apiary	16	0

Source: General Diretion of Food and Veterinary (DGAV-Direção Geral de Alimentação e Veterinária)

The European Union supports research projects in the field of bee health through the Seventh Framework Programme, besides the European Commission, supports the action COST COLOSS “, establishing a network of scientists,_veterinarians, students and beekeepers, over 60 countries, to follow the evolution of colony losses and join forces with participating in national research programs.

The World Organisation for Animal Health recently issued a statement on the health of bees and submitted a proposal towards the international community to increase research on the causes of bee mortality and to improve control and combat the many already known and emerging diseases".

The Commission is working with the World Organisation for Animal Health, to exchange scientific information on the bees health and no more in future to have actions that overlap.

BIBLIOGRAPHY

(1) - Implementing order Nº 27/2010, laying down additional rules on the implementation of the National Beekeeping Programme (NAP).

(2) - Law Decree nº 106/2010, October 1 st, concerning authorizations and conditions of use for seed treatment with a view to the protection of non-target organisms, in particular bees of plant protection products containing the active substance clothianidin, thiamethoxam, fipronil and imidacloprid. "

(3) PNPR 2011- Results, the General Directorate of Food and Veterinary "Veterinary drug residues in honey", Master's thesis in Food Safety, by Adriana de Jesus Inácio Belas, Faculty of Veterinary Medicine.

(4) Publication of the Regional Direction of Agriculture of Entre Douro e Minho, on the progress and status of beekeeping in Portugal, 2006

(5) "Bee health", Communication from the Commission to the European Parliament, the European Council, Brussels, 06-12-2010.

(6) ASAE´s website: " Legislação, Saúde Pública e Segurança alimentar, Mel".

(7) - EFSA´s website, " Interaction between pesticides and other factors in effects on bees" ": http://www.efsa.europa.eu/en/supporting/pub/340e.htm

(8) - EFSA´s website: " Bee health": http://www.efsa.europa.eu/en/topics/topic/beehealth.htm

Xenobiotics in honey in Portugal

Belas, A., Epifânio, A.F., Almeida, C., Carrapiço, B., Vaz, Y., São Braz, B.

CIISA Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Portugal.

Introduction

Honey is a very complex biological product, produced by bees of the genus *Apis*, being the most important product of beekeeping, marketed worldwide. It has low water content and its composition includes a high content of carbohydrates, proteins, amino acids, vitamins such as niacin, riboflavin, pantothenic acid, B complex, C, D and E. The mineral content of honey ranges between 0.1 and 0.5 %, with potassium being the most abundant mineral. This product is appreciated for its characteristic taste, its nutritional value and also due to an increased interest in natural and healthy products for the consumers. Honey is used as a sweetener, being consumed due to its beneficial effects, especially by children and seniors.

Even being considered a natural product, honey production is carried out in a more or less polluted environment and uses materials and products susceptible to contaminate it. The residues of xenobiotics that may be present in honey (pesticides, antibiotics and heavy metals) can came from the environment (agricultural or environmental sources) or from beekeeping practices (products for treatment of bee diseases, beehive materials, contaminated wax, shields of wood, extraction equipment) (Bogdanov, 2006, 2008). These residues may compromise product quality and safety, causing public health problems and interference in trade.

Pharmacologically active substances residues of VMPs (Veterinary Medicine Products)

In beekeeping, the use of antibiotics and synthetic acaricides are a common practice for the treatment of bacterial and parasitic diseases of bees and can result in their presence in honey. To avoid the presence of residues of pharmacologically active substances of VMPs in bee food only veterinary drugs authorized by the competent authorities (Direção-Geral de Alimentação e Veterinária [DGAV], in Portugal) should be used. The use of veterinary medicines is not recommended immediately before and during the harvest of honey, and if it's done the honey should not be used for human consumption (FAO /WHO, 2010).

A "zero tolerance" policy to the presence of antibiotic residues in honey is applied in Europe, but it depends on the analytical methodologies available for the identification of substances and the determination of its concentration in the product. The "zero tolerance" concept applies essentially to substances identified and suspected to have carcinogenic or mutagenic properties, in accordance with Commission Regulation (EC) No 37/2010 on "Pharmacologically active substances and their classification regarding maximum of residues in food of animal origin". Thus in the European Union the use of antibiotics in beekeeping is not authorized, has there are no definition for the maximum residue of antibiotics in honey. The identification of bee products contaminated with antibiotics implies its destruction and the application of a penalty to the producer.

Acaricides are use in agriculture against pests (plant protection products) and in beekeeping are used to control the mite *Varroa destructor*. The use of these pesticides (synthetic or organic) is widespread. They are easily applied, act by direct contact and do not require specific knowledge about the biology of bees (FAO/WHO, 2010). In Portugal the pharmacological active substances authorized as veterinary medicines are coumaphos, flumethrin, tau-fluvalinate, amitraz and thymol (organic acaricide).

Heavy metals

The contamination of honey with heavy metals can occur by natural or man-made causes. Natural causes are associated with rocky erosion and volcanic activity. Among the anthropogenic causes there are the inadequate application of fertilizers in agriculture, forest fires and increasing air pollution.

Soil contamination by cadmium can be due to the application of phosphate fertilizers (European Food Safety Authority [EFSA], 2009), followed by the burning of fossil fuels, the production of iron and steel, of nonferrous metals, cement , other materials rich in cadmium (paint pigments, plastics) and by waste incineration (Autoridade de Segurança Alimentar e Económica [ASAE], 2009).

The use of fungicides rich in copper, as copper sulfate in crops such as apple, grapevine, potato and peach, can be responsible for soil contamination by copper, and neighboring hives. Other sources of copper pollution are fertilizers, algaecides containing copper, the incineration of municipal waste, metal smelting and mining activity (World Health Organization [WHO], 1998).

Incorrect beekeeping practice can also lead to the introduction of chemical contaminants in honey, for example through the paint and varnish of hives, the poor sanitation of transport vehicles and plants where honey is extracted and prepared, and the use of utensils of unsuitable materials. In the processes of transportation, extraction and packaging of honey good hygiene practices should apply in accordance with Regulation (EC) No 852/2004 of the European Parliament and of the Council, April 29. The national law also establishes that apiaries location shall not be less than 50 meters from the public traffic route and not less than 100 meters from any building in use (DL No. 203/2005, November 25).

Maximum levels for contaminants in foodstuffs including maximum levels of Cd and Pb are established in Regulation (EC) No 1881/2006 of the European Parliament and of the Council of 19 December. However, for honey, no ceilings are established in the EU or national law, but several studies have already demonstrated the presence of Cd and Cu in honey from different sources (Fredes & Montenegro, 2006; Frías *et al*., 2008; Stankovska *et al*., 2008).

Control of residues of xenobiotics in honey

The control of residues of pharmacologically active substances and contaminants, both for products from EU Member States, as well as for imported foods from third countries, including the honey, has been mandatory since 1997, as established by Council Directive 96/23/EC, 29 April, on "Measures to monitor certain substances and residues thereof in live animals and animal products", by Decision 97/747/EC, 27 October, fixing the "Levels and frequencies of sampling provided for in Directive 96/23/EC" and by Commission Decision 98/179/EC, 23 February, laying down "Detailed rules for official sampling procedures and official treatment of samples until they reach the laboratory responsible for analysis".

In Portugal, the detection of residues in honey is made in accordance with the National Residue Control Plan (PNCR), which is implemented and coordinated by the official veterinary services DGAV. Table 1 presents the results of monitoring of residues in honey by PNCR between 2006 and 2011. All samples analyzed for the subgroup B3c were within the authorized limits; heavy metals were not detected in any sample.

Table 1 - Honey samples analyzed between 2006 and 2011 by National Residue Control Plan (DGAV, 2006 a 2011)

Year	**Number of samples collected per group**						**Non-compliant samples**
	A6	B1	B2c	B3a	B3b	B3c	
2011	-	22	24	16	-	-	0
2010	9	34	28	15	15	15	1 (B1)
2009	10	32	32	20	0	20	0
2008	10	37	33	21	21	42	1 (B1)
2007	6	7	18	5	3	15	0
2006	11	22	5	10	6	8	0

Legend: A6 - substances listed in Annex IV of Regulation (EC) No 2377/90; B1 - antibacterial substances including sulfonamides and quinolones; B2c - pyrethroids; B3a - organochlorines including PCBs; B3b - organophosphorus compounds; B3c - chemical elements (metals heavy).

Given the need to control honey, the availability of specific, sensitive, rapid and relatively inexpensive analytical methodologies is essential in order to provide beekeepers with quality assurance for the honey being marketed, since excessive use or misuse of pharmacologically active substances can affect honey quality and impair their export.

In these studies the authors tried to develop analytical methodologies applicable to the evaluation of the presence of residues of pharmacologically active substances in honey samples from various regions/districts of Portugal, namely Tetracyclines (tetracycline and oxytetracycline), sulphamide (sulfathiazole, sulfamethoxazole) and acaricides; and also contaminants as cadmium and copper.

Materials and methods

To assess the presence of residues of pharmacologically active substances (tetracyclines, sulfonamides and acaricides) 42 samples were obtained from the national market, and from producers, from different regions of Portugal (Norte, Centro, Lisboa and Vale do Tejo, Alentejo, Algarve and Açores). For the presence of heavy metals (Cd and Cu) 21 honey samples were investigated from 7 districts of Portugal (three samples per district: Coimbra, Castelo Branco, Santarém, Évora, Setúbal, Beja and Faro). All samples were randomly collected with respect to the sales outlet, the batch or the producer. Samples were kept in the original containers at room temperature and protected from light until laboratory processing.

For the detection of pharmacologically active substances residues, and as a screening method, we used a solid phase extraction followed by Thin Layer Chromatography using different elution systems and different visualization reagents for each chemical group. In the case of synthetic acaricides (coumaphos, tau-fluvalinate and flumethrin) gas chromatography with electron capture detector was also used.

Determination of heavy metals (Cd and Cu) was performed by atomic absorption spectrophotometry after dry digestion process and quantification was carried out in an atomic absorption spectrophotometer (Perkin Elmer Analyst 700) with flame burner air / acetylene (17/2 ml / min), hollow cathode lamps as a source of emission and wavelength of 228.8 nm to 324.8 nm for Cd and Cu.

Results and discussion

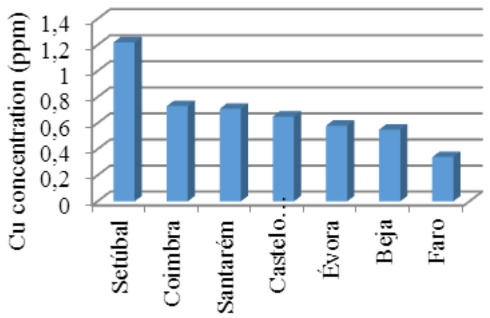
Results obtained indicate that 9.5% of Portuguese honey samples analyzed (n = 42) were suspected for the presence of antibiotics, specifically sulfatiozol (Table 2). Tetracycline and synthetic acaricides were not detected (Belas, 2012).

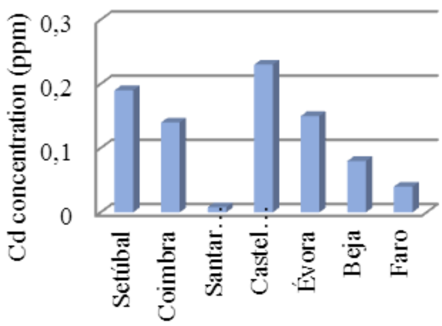
Table 2 - Determination of pharmacologically active substances residues in Portuguese honey samples (*n=42*).

Region	Samples (n)	Positive samples	Substance detected
Norte	5	0	-
Centro	12	3	Sulfatiazol
Lisboa e Vale do Tejo	11	0	-
Alentejo	6	1	Sulfatiazol
Algarve	5	0	-
Região Autónoma dos Açores	3	0	-

Results obtained on the determination of Cu and Cd in portuguese honey samples are presented in Figures 1 and 2 (Epifânio, 2012). The mean concentrations for Cu were: Setúbal district 1.22 ± 0.24 ppm, Coimbra district 0.73 ± 0.28 ppm, Santarém district 0.71 ± 0.89 ppm, district of Castelo Branco 0.65 ± 0.44 ppm, Évora district 0.58 ± 0.35 ppm, Beja district 0.55 ± 0.19 ppm and the district of Faro 0.34 ± 0.28 ppm. Results obtained in the samples analyzed for Cd were: district of Castelo Branco 0.23 ± 0.06 ppm, Setúbal district 0.19 ± 0.04 ppm; district of Évora 0.14 ± 0.15 ppm, Coimbra district 0.14 ± 0.13 ppm, Beja district 0.07 ± 0.06 ppm. Results for Faro and Santarém samples are below the detection limit. The average concentrations of Cd and Cu obtained from different districts have no statistically significant difference between them ($p > 0.05$).

Figure 1 and 2 - Cu (ppm) and Cd (ppm) concentrations in samples of honey from different Portuguese geographic origins





The risk arising from the presence of pharmacologically active substances residues in Portuguese honey is not considered high and can be reduced by improving the health status of the bee population, the training of beekeepers for a correct use of approved drugs and effective monitoring and veterinary control.

The presence of heavy metals in honey and food in general is of high importance, since their bio accumulative effects may put consumers' health at risk. This study showed that Cd and Cu are present in some honey samples probably due to contamination from industrial activity or negligent beekeeping practices. So it seems that the protection of apiaries from contamination sources (highways, industries) should be strengthened.

These studies, as well as many others, warn to the possible change in the composition of foods considered natural and safe. In this particular case it is important to protect the natural features of honey and minimize its contamination with residues and therefore minimize negative impact in public health.

References

Autoridade de Segurança Alimentar e Económica (2009). Perfil de risco dos principais, alimentos consumidos em Portugal. Acedido em Jan. 3, 2012, disponível em http://www.fipa.pt/userfiles/file/i005411.pdf.

Belas, A. J. I. (2012). Resíduos de Medicamentos Veterinários em Mel. Dissertação de Mestrado em segurança alimentar. Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa.

Bogdanov, S. (2006). Contaminants of bee products. Apidologie, 37: 1-18.

Bogdanov, S., Jurendic, T., Sieber, R. & Gallmann, P. (2008). Honey for nutrition and health: a review. Journal of American College of Nutrition, 27(6), 677-689.

Commission Decision nº 97/747/CE, 27 October 1997 - Levels and frequencies of sampling provided for in Directive 96/23/EC. Accessed Fev, 2011, at: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31997D0747: PT:HTML

Commission Decision nº 98/179/CE, 23 February 1998 - Detailed rules for official sampling procedures and official treatment of samples until they reach the laboratory responsible for analysis. Official Journal of the European Community, L65/31.

Commission Regulation nº 37/2010, 22 December 2009 - Pharmacologically active substances and their classification regarding maximum of residues in food of animal origin. Official Journal of the European Union, L15 de 20.01.2010, 1-72.

Council Directive 96/23/CE, 29 April 1996 - Measures to monitor certain substances and residues thereof in live animals and animal products. Official Journal of the European Community, L 302.

Decreto-lei nº 148/99 de 4 de Maio de 1999. Relativo às medidas de controlo a aplicar a certos subprodutos e aos seus resíduos em animais vivos e respectivos produtos. Diário da República, nº 103, série I-A

Direção-Geral de Alimentação e Veterinária (2006-2011). Plano nacional de controlo de resíduos – relatório anual 2006, 2007, 2008, 2009, 2010 e 2011. Acedido em Jan 7, 2014, disponível em http://www.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?actualmenu=187909&generico=19631&cboui=19631.

Epifânio, A.F.R.P. (2012). Determinação de metais pesados em mel nacional por espectrometria de absorção atómica. Dissertação de Mestrado em segurança alimentar. Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa.

European Food Safety Authority (2009). Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on cadmium in food. The EFSA Journal, 980, 1-139.

European Food Safety Authority (2010). EFSA panel on contaminants in food chain (CONTAM); Scientific opinion on lead in food. The EFSA Journal, 8(4), 1570-1717.

FAO/WHO (2010). Discussion paper on veterinary drugs in honey production. Acedido em Ago.22, 2011, disponível em: ftp://ftp.fao.org/codex/ccrvdf19/rv19_10e.pdf

Fredes, C. & Montenegro, G. (2006). Heavy metals and other trace elements contents in Chilean honey. Ciencia e Investigación Agraria, 33(1), 50-58.

Frías, I., Rubio, C., González-Iglesias, T., Gutiérrez, A. J., González-Weller, D. & Hardisson, A. (2008). Metals in fresh honeys from Tenerife Island, Spain. Bulletin of Environmental Contamination and Toxicology, 80, 30-33.

Stankovska, E., Stafilov, T. & Šajn, R. (2008). Monitoring of trace elements in honey from the Republic of Macedonia by atomic absorption spectrometry. Environmental, Monitoring and Assessment Journal, 142, 117-126.

HACCP implementation in a small scale honey production: the case of honey Alombada

Ana Neves[1], José Francisco Silva[1], Manuel A. Coimbra[2]
1 – Latina Pastry
2 – QOPNA, Chemistry Department, of Aveiro University

1. Introduction

Honey is a natural substance produced by bees of the species *Apis mellifera*. Honey is mainly composed by a complex mixture of carbohydrates, of which fructose and glucose correspond to approximately 85-95% of its composition. Other minority substances of honey are organic acids, amino acids, proteins, minerals, vitamins, and lipids[1]. It is a natural product of high nutritional value and is considered low risk product due to its high sugar content, low pH, and low water content. However, its handling may increase the hazards, mainly the physic ones, justifying the existence of a good practice code and HACCP plan application to ensure food quality and safety.

The criteria of honey quality are specified by the Council Directive 2001/110/EC[2] and by the Portuguese Decreto-Lei n.º 214/2003[3], ruling the sensory, chemical, physical, and microbiological characteristics.

The purpose of the present work is to: i) address the physical and chemical characteristics of honeys in general, taking in to account the current legislation, ii) explore the honey production mode, identifying the inherent risks, and iii) present the prerequisites and HACCP plan to be implemented for a product from a small producer: the Honey Alombada.

2. Honey physical and chemical characteristics and legislation

Honey is a saturated solution of fructose and glucose. According to the Portuguese legislation, the minimum content of fructose and glucose in honey is 60 g/100g and the maximum content of sucrose is 5 g/100g[3]. Disaccharides such as maltose, trehalose, and isomaltose, trisaccharides such melezitose[4], and oligosaccharides, are all present in very small amount. The honey sugar composition has been reported to be dependent on the type of flowers visited by bees as well as the edaphoclimatic conditions[5].

The maximum water content allowed in honeys is generally 20%, except in heather honey (*Calluna* spp.), that is 23%[3]. The water content is a key parameter to establishing the honey shelf life[6]. Honeys with a high water content, more than 20%[7], tend to split into two phases: a bottom granulated phase and a liquid one on top of the container. Consequently, the top liquid phase contains a high free water content, being the water activity (a_w) greater than 0.6 (the honey a_w ranges between 0.5 e 0.6[8]). This allows yeasts development, causing honey spoilage by fermentation[9]. The water content depends on factors involved in the ripening phase of the honey, such as: i) climatic conditions, ii) water content of the original nectar, iii) harvest season, iv) maturity degree achieved in the beehive[11,12], and v) storage conditions due to honey higroscopicity[10,13].

The content of water insoluble matter, suspended wax particles, and/or residues of insects or vegetables in honey, according to the Portuguese legislation, shall not exceed 0.1 g/100g of honey, with exception of pressed honey, whose maximum value is 0.5 g/100g of honey[3].

The electrical conductivity legislated for honeydew honey (natural sweet substance produced by bees of the species *Apis mellifera* from secretions of living parts of plants or secretions of plant sucking insects), chestnut flower honey, and the mixture of these two is 0.8 $mS.cm^{-1}$, at minimum. For the remaining honeys or their mixture, the electrical conductivity is 0.8 $mS.cm^{-1}$, at maximum[3]. This parameter is closely related to the concentration of mineral salts, organic acids. and proteins, presenting a large variability according to the floral origin[14].

The acidity of the honey is due to the presence of organic acids, mainly gluconic acid, in equilibrium with its lactones[15]. The acidity variation between different types of honey may be attributed to the variation of these constituents which range according to extraction period[16], as well as floral type[6]. Organic acids, which constitute 0.57% of honey[17], can be: i) spoilage indicators due to acetic acid production[6]; ii) aging indicators, as the storage conditions can result in honey fermentation and, consequently, to acetic acid production; and iii) authenticity and purity indicators, such citric, fumaric, malic, and succinic acids[18]. Under the Portuguese legislation, the maximum amount of free acids allowed in all honeys, except in honey for industrial use, is 50 miliequivalents of acids in 1 kg of honey ($meq.kg^{-1}$)[3].

Although not legislated, honey pH ranges between 3.4 and 6.1, with an average value of 3,9[8]. However, honey pH is not directly related to its free acidity due to the buffering effect of the several acids and minerals present in honey[6].

In accordance with the Portuguese legislation, the hydroxymethylfurfural content (HMF) and diastasic index (ID, enzymatic hydrolysis of starch and glycogen into maltodextrins) are parameters that should be determined after processing and honeys mixture, if these events take place[3]. Regarding the HMF content, this is a parameter that is related with the lower freshness of honey since it is not present in fresh honey and tends to increase during the product processing and/or storage. The HMF can be formed by hexoses dehydration in acidic medium or through Maillard reaction[14]. Its concentration is influenced by: i) temperature and time of processing, ii) storage conditions, iii) pH, and iv) floral source[1]. The maximum content of HMF allowed is 40 $mg.kg^{-1}$ for general honeys, with the exception for honey intended for industrial use and honeys whose origin is declared as from tropical regions or mixtures containing honeys from these origins (maximum content of HMF is 80 $mg.kg^{-1}$)[3]. If HMF levels are higher than those allowed, it indicates that the honey was submitted to overheating and/or poor storage practices. On the other hand, as well as the HMF content, diastase activity may be used as an aging and overheating indicator, as the activity of this honey natural enzyme decreases in both situations[20]. However, the diastase activity should be carefully used due to its wide variability. The diastase activity levels depend on floral and geographical origin of honey, as well as its freshness[1].

According to the Portuguese legislation, the diastasic index is measured using the Schade scale. In this scale, a Schade unit, or a diastase activity unit, is defined as the amount of enzyme which hydrolyses 0.01 g of a cooked starch solution, during one hour, at 40ºC[21,22]. To the general honeys, the diastasic index must be, at minimum, 8 Schade units. The exceptions are the honeys intended for industrial use and honeys with low enzymes content (for example, citrus honeys) and with HMF content not higher than 15 $mg.kg^{-1}$, and with diastasic index of 3 Schade units, as minimum[3].

According to the Regulation (EU) n.º 1169/2011[23], the mandatory information in honey labeling is the following: i) product name, ii) country of origin, iii) batch/lot of the product, iv) number of veterinary control, v) net weight, vi) date of minimum durability or the "use by" date, vii) special storage conditions, viii) nutritional information, and ix) name or business name and address of food manufacturer.

3. Honey production mode

The honey production, the equipment to be used, and the steps followed in the processing depend on the operation scale. There are several steps common to all honey productive processes, namely the uncapping (removal of the operculum, a thin layer of wax secreted by bees), the frames centrifugation, and the honey pre-filtering, decantation, filtering, and homogenization (figure 1).

The major difference between the production mode of small producers and the industrial level is related to the use of heating and subsequent cooling, characteristic of the industrial process. The thermal processing of honey eliminates the microorganisms that are responsible for its spoilage, reduces the moisture content to levels that slow the fermentation process, modifies the tendency of the honey to granulate, and facilitates honey filling by reducing its viscosity. Therefore, during manipulation and packaging, honey is usually submitted to thermal processing[7]. The use of high temperatures in honey processing – above 100ºC[17] – are not accepted as current practices for honey quality standards[7].

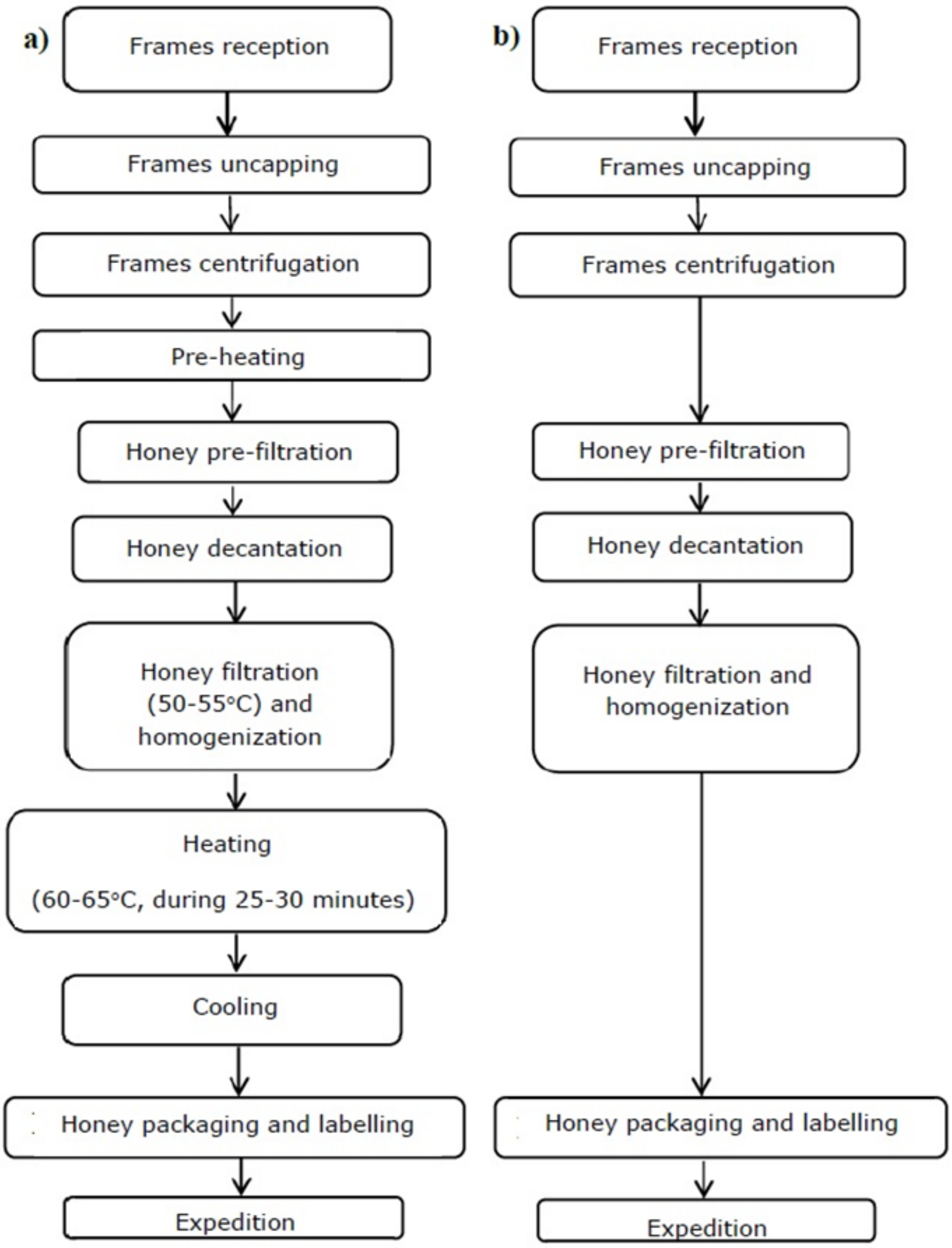


Figure **1.** Flow diagram of honey production at: **a)** industrial level[24] and **b)** small producers.

4. Hazards identification associated to honey production

The hazards associated to honey production are closely related with the production spaces as well as with the equipments and instruments used to handle the honey. Therefore, some hazards may be highlighted, such as the: i) use of pesticides on crops/ vegetation that surround the apiaries (chemical hazard), ii) use of artificial feeding into hives, in winter, which can lead to the occurrence of fermentations due to high temperatures and humidity (microbiological hazard), and iii) practices adopted in the moment of honey harvesting – collection of frames full with honey of the field – to be carried out with some precautions in order to avoid high water and HMF contents (chemical hazards) in honey, as well as *C. botulinum* spores (microbiological hazard). The hazards arising from the activities performed at the honey plant are mainly the physical ones, associated to the equipment and instruments used to process the honey.

5. Case Study: the Honey Alombada

The honey Alombada (figure 2) is produced in apiaries located in mountain areas (figure 3) of Macinhata and Valongo do Vouga, Águeda, in Portugal. These apiaries are located in PEFC (Programme for the Endorsement of Forest Certification) forests and, allied to this requirement, the honey Alombada production mode is being converted into an organic production mode. In the beekeeping case, the legislation associated to the organic mode of production focuses mainly on two aspects: i) minimization of the use of pesticides and other chemical products on crops/vegetation where apiaries are installed and ii) minimization of the use of chemically synthesized allophatics used in the treatment of bee pathologies arising in the hives. Therefore, additional prerequisites concerning to foodstuffs hygiene, will be referred briefly, as they are the ruled by the Regulation (EC) n.º 852/2004[25].

Figure 2. Honey Alombada.

Figure 3. Apiaries for honey Alombada production.

5.1. Prerequisites

The prerequisites that provide the basis for the HACCP system implementation have a preponderant role for the agro-food industry. This is particularly relevant to organic production of honey Alombada. The additional prerequisites are presented in the Regulation (EC) n.º 889/2008[26], and concern to the organic production mode, which are related to the: i) installations and materials that constitute the apiaries, ii) bee pathologies and their treatments, and iii) artificial feeding application.

5.2. HACCP plan implementation

In the HACCP plan outlined for honey Alombada the 12 steps mentioned in *Codex Alimentarius*[27] were followed. The hazards associated with each step of the production process associated to this specific honey were analyzed. All identified hazards of the different stages of honey Alombada production were subjected to a risk matrix and decision tree. It was detected a single Critical Control Point (CCP), related to the application of artificial feeding (microbiological hazard) since its application within the hives, whose temperatures are around 35ºC, associated with heavy rainfall of the winter can propitiate fungi growth. Although the presence of fungi in honey is not a severe risk for human consumption, a high content of these microorganisms provides product degradation by fermentation, which can be sensory noticed. In accordance with the HACCP plan, the application of artificial feeding in beehives requires that the beekeeper replace it each two days, as a preventive measure. According to the monitoring procedures for the identified CCP, the beekeeper is responsible for performing a visual inspection of the beehives where the artificial feeding was applied, evaluating the possible presence of fungi. This practice should be done also every two days and should be registered the days of application and the day of removal of the artificial feeding, as well as the absence/presence of fungi in removal day. In the eventual presence of fungi, it should be adopted corrective actions such as the replacement of the frames containing fermented syrup and, in more severe cases of non-compliance, the rejection of the product.

6. Conclusions

Honey is a "safe" food product due to its intrinsic characteristics such as the high sugar content, low water activity, and low pH.

The use of good practices upstream of honey production is important to obtain a high quality product fulfilling the food safety requirements. The beekeeper assumes a preponderant role since he is responsible for the practices adoption to ensure the health of beehives. Hence, the beekeeper must have the appropriate training to perform this activity.

Through the HACCP plan defined for honey Alombada it was possible to identify a single CCP which is associated with the artificial feeding application. However, because the artificial feeding is only used if the bees do not have honey reserves for winter, this CCP is not always a concern. As long as the prerequisites associated with the honey production are implemented and fulfilled, certainly, the honey is not a hazard food.

7. Bibliography

1. Gomes, S., Dias, L. G., Moreira, L. L., Rodrigues, P., and Estevinho, L. (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal, *Food and Chemical Toxicology*, 48:544-548
2. European Union (UE) (2001). Council Directive 2001/110/EC relating to honey. Official Journal of the European Communities
3. Decreto-Lei n.º 214/2003, de 18 de setembro. *Diário da República n.º 216/2003 – I Série A*. Ministério da Agricultura, do Desenvolvimento Rural e das Pescas. Lisboa
4. Mendes, E., Brojo Proença, E., Ferreira, I. M. P. L. V. O., and Ferreira, M. A. (1998). Quality evaluation of Portuguese honey, *Carbohydrate Polymers*, 37:219-223
5. Zamora, M. C., and Chirife, J. (2006). Determination of water activity change due to crystallization in honeys from Argentina, *Food Control*, 17:59-64
6. Ojeda de Rodríguez, G., Ferrer, B. S., Ferrer, A., and Rodriguez, B. (2004). Characterization of honey produced in Venezuela, *Food Chemistry*, 84(4):499-502
7. Tosi, E., Ré, E., Lucero, H., and Bulacio, L. (2004). Effect of honey high-temperature short-time heating on parameters related to quality, crystallization phenomena and fungal inhibition, *LWT – Food Science and Technology*, 37:669-678
8. Iurlina, M. O., and Fritz, R. (2005). Characterization of microorganisms in Argentinean honeys from different sources, *International Journal of Food Microbiology*, 105:297-304
9. Gleiter, R. A., Horn, H., and Isengard, H.-D. (2006). Influence of type and state of crystallization on the water activity of honey, *Food Chemistry*, 96:441-445
10. Chirife, J., Zamora, M. C., and Motto, A. (2006). The correlation between water activity and % moisture in honey: Fundamental aspects and application to Argentine honeys, *Journal of Food Engineering*, 72 (3):287-292
11. Pires, J., Estevinho, M. L., Feás, X., Cantalapiedra, J., and Iglesias, A. (2009). Pollen spectrum and physic-chemical attributes of heather (Erica sp.) honeys of north Portugal, *Journal of the Science of Food and Agriculture*, 89:1862-1870
12. Finola, M. S., Lasagno, M. C., and Marioli, J. M. (2007). Microbiological and chemical characterization of honeys from central Argentina, *Food Chemistry*, 100:1649-1653
13. Ball, D. W. (2007). The Chemical Composition of Honey, *Journal of Chemical Education*, 84(10):1643-1646
14. Terrab, A., Díez, M. J., and Heredia, F. J. (2003). Palynological, physico-chemical and colour characterization of Moroccan honeys. II. Orange (*Citrus* sp.) honey, *International Journal of Food Science and Technology*, 38(4):387-394
15. Bath, P. K., and Singh, N. (1999). A comparison between *Helianthus* and *Eucalyptus lanceolatus* honey, *Food Chemistry*, 67(4):389-397
16. Singh, N., and Bath, P. K. (1997). Quality evaluation of different types of Indian honey, *Food Chemistry*, 58(1-2):129-133
17. Aurongzeb, M., and Kamran Azim, M. (2011). Antimicrobial properties of natural honey: a review of literature, *Pakistan Journal of Biochemistry and Molecular Biology*, 44(3):118-124
18. Suárez-Luque, S., Mato, I., Huidobro, J. F., and Simal-Lozano, J. (2002). Solid phase extraction procedure to remove organic acids from honey, *Journal of Chromatography B*, 770(1-2):77-82
19. Tosi, E., Ciappini, M., Ré, E., and Lucero, H. (2002). Honey termal treatment effects on hydroxymethylfurfural content, *Food Chemistry*, 77:71-74
20. Bogdanov, S., The Book of Honey: a short history of honey. Bee Product Science, chapter 5, August, 2009. Disponível em: www.bee-hexagon.net, acedido em novembro de 2012
21. Bogdanov, S., Martin, P., Lüllmann, C., Borneck, R., Flamini, Ch., Morlot, M., Heretier, J., Vorwohl, G., Russmann, H., Persano-Oddo, L., Sabatini, A. G., Marcazzan, G. L., Marioleas, P., Tsigouri, K., Kerkvliet, J., Ortiz, A., and Ivanov, T. (1997). Harmonised methods of the European honey commission, *Apidologie* (extra issue), 1-59
22. Bogdanov, S. (2002). Harmonized methods of the International Honey Comission, Swiss Bee Research, FAM, Libefeld, CH-3003 Ber, Switzerland
23. Regulation (EU) n.º 1669/2011 of the European Parliament and of the Council, of 25 October 2011, on the provision of food information to consumers, amending Regulations (EC) n.º 1924/20066 and (EC) n.º 1925/2006 of the Parliament and of the Council, and repealing Comission Directive 87/250/EEC, Council Directive 90/496/EEC, Comission Directive 1999/10/CE, Directive 2000/13/EC of the European Parliament and the Council, Comission Directives 2002/67/EC and 2008/5/EC and Comission Regulation (EC) n.º 608/2004
24. Subramanian, R., Hebbar, H. U., and Rastogi, N. K. (2007). Processing of Honey: a review, *International Journal of Food Properties*, 10:127-143
25. Regulation (EC) n.º 852/2004 of the European Parliament and of the Council, of 29 April 2004, on the hygiene of foodstuffs. 2004, European Parliament and of the Council: Official Journal of the European Union
26. Comission Regulation (EC) n.º 889/2008, of 5 September 2008, laying down detailed rules for the implementation of Council Regulation (EC) n.º 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. 2008, Comission: Official Journal of the European Union
27. *Codex Alimentarius* (2003). Recommended International Code of Practice General Principles of Food Hygiene. CAC/RCP 1-1969, Rev. 4-2003

Antioxidant activity of commercial honeys: influence of floral origin

Sónia Soares[a], Anabela SG Costa[a], Liliana Ribeiro[a,b], Diana Nascimento[a,b], Luís M. Cunha[b], M. Beatriz P. P. Oliveira[a]
[a] REQUIMTE, Dep. Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Portugal; [b] REQUIMTE, Faculdade de Ciências da Universidade do Porto, Porto, Portugal
**email: beatoliv@ff.up.pt*

Nowadays, there is a high consumer demand for natural food products, mainly due to increased information and awareness of possible risks associated to diet. The quest for convenience, authenticity, quality and safety, as well as the growing concerns with the environment and health has made natural and organic products, rich in antioxidants, a target of growing interest for consumers.

Honey is included in this group of food products since it consumption is related to the decreasing risk of certain diseases (coronary heart disease, cancer, cataracts, inflammation, and other diseases/disorders). Furthermore, the use of honey as ingredient, in certain foods, prevents its deterioration (for example enzymatic browning of fruits and vegetables and lipid oxidation of meat) (Arraez-Roman *et al*., 2006).

Honey is defined as a natural sweet substance produced by honey bees (*Apis mellifera*) from the nectar of plants (blossom honey or nectar honey) or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants (honeydew honey), which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (DL nº 214/2003 of 18th September).

Honey from plant nectar can be classified as monofloral, when arising predominantly from a single botanical origin (acquiring its sensory, physicochemical and microscopic features), or multifloral honey, from different floral origins (Normative Instruction nº11, of 20th October of 2000). The most common monofloral honeys in Portugal are heather (up to 45 % of pollen from *Erica sp*.), eucalyptus (up to 70 % of pollen from *Eucalyptus sp*.) and rosemary (minimum of 15 % of *Lavandula sp*. pollen). Monofloral honeys, due to its refined flavor and taste, are the most appreciated by consumers and have a higher commercial value than multifloral honeys (Maia, 2013). According to the *Codex Alimentarius*, honey for human consumption should not have added any food ingredient, including food additives or any substance other than honey, and should be free of foreign organic or inorganic materials. Honey composition varies according to several factors such as the floral source, climate, environmental and seasonal conditions, as well as handling and processing (Al-Mamary *et al*., 2002; Anklam, 1998; Arraez-Román *et al*., 2006; Azeredo *et al*., 2003; Baltrušaitytė *et al*., 2007; Küçük *et al*., 2007). In some cases, climate variations and different geographical origins are inducing change factors of honeys from the same floral origin (Anklam, 1998).

Honey is composed mainly by carbohydrates (glucose, fructose, maltose, and sucrose), water and other bioactive molecules, as enzymes, amino acids, phenolic compounds, flavonoids, ascorbic acid, organic acids, pigments, pollen and traces of wax (Al-Mamary *et al.*, 2002; Baltrušaitytė *et al*., 2007; Bertoncelj *et al*., 2007). The honey antioxidant properties are due to the presence of flavonoids and phenolic compounds, justifying also some therapeutic action of this natural product. According to Al-Mamary *et al*., (2002) and Anklam (1998), the antioxidant capacity of honey and the content of bioactive compounds depend mainly of its floral source.

Several studies have shown that, in general, dark honeys with higher water contents have an increased antioxidant activity (Chen *et al*., 2000; Frankel, 1998; Gheldof and Engeseth, 2002; Nagai *et al*., 2001; Aljadi and Kamaruddin, 2004).

The importance of phenolic compounds has increased considerably in recent years, due to their ability to eliminate free radicals, entities responsible for the attack and damage of vital molecules to life. Besides its beneficial effect, phenolic compounds may also be useful for the assessment of the geographical and floral origin of honeys. Some studies have shown that hesperidin can be considered a marker of citrus honey (Ferreres *et al*., 1994; Ferreres *et al*., 1998), kampferol of rosemary honey (Ferreres *et al*., 1994; Tomas-Barberan *et al*., 2001), ellagic acid of heather honey (Cherchi *et al*., 1994; Ferreres *et al*., 1996a; Ferreres *et al*., 1996b), cinnamic acids for chestnut honey (Cherchi *et al*., 1994) and luteolin, kaempferol, quercetin and kampferol for eucalyptus honey (Martos *et al*., 2000a; Martos *et al*., 2000b).

In this study, we evaluated the antioxidant activity and the levels of related bioactive compounds in honey samples from different floral origins.

Material and methods

In this work, 18 honey samples from different floral origins, were acquired in supermarkets and producers. Table 1 describes the analyzed samples, detailing the respective names and floral origins.

Table 1 - Samples description with respective floral origin

Sample	Floral Origin
Heather 1 [1]	Heather
Heather 2 [1]	Heather
Heather 3 [1]	Heather
Heather 4 [1]	Heather
Heather 5 [1]	Heather
Heather 6 [2]	Heather
Heather PDO 1 [2]	Heather
Rosemary PDO 1 [2]	Rosemary
Rosemary PDO 2 [2]	Rosemary
Rosemary 1 [2]	Rosemary
Rosemary and linden [2]	Rosemary and linden
Rosemary and almond blossom [2]	Rosemary and almond blossom
Orange [1]	Orange
Eucalyptus [2]	Eucalyptus
Blueberry [2]	Blueberry
Chestnut [2]	Chestnut
Multifloral[1]	Multifloral
Multifloral PDO 1 [2]	Multifloral

[1]Commercial honey; [2] Producer honey

For the bioactive compounds evaluation, spectrophotometric methods were used. Total phenolic compounds were determined according to Alves *et al.* (2010) with minor modifications. Its quantification was estimated using a calibration curve of gallic acid (R^2=0.9991). The determination of total flavonoids was performed following Barroso *et al.* (2011), with some minor modifications. Their estimation was achieved using a calibration curve of epicatechin (R^2=0.9999).

The evaluation of the antioxidant activity was carried out using two assays: the radical 1,1-diphenyl-2-picrylhydrazyl ($DPPH^•$) inhibition capacity and the antioxidant power by reducing the ferric ion (FRAP) according to Benzie and Strain (1996). The antioxidant activity by the $DPPH^•$ assay was achieved according to Meda *et al.*, (2005) with some modifications, by calculating the inhibition percentage at a concentration of 100 mg honey/mL solution. The determination of the antioxidant activity by the FRAP method was performed using a calibration curve of ferrous sulphate (R^2=0.9982).

Results and discussion

The results for the different honey samples and the influence of its floral origin are presented in Figures 1, 2, 3 and 4 (total phenolic compounds, total flavonoids, and $DPPH^•$ and FRAP methods, respectively).

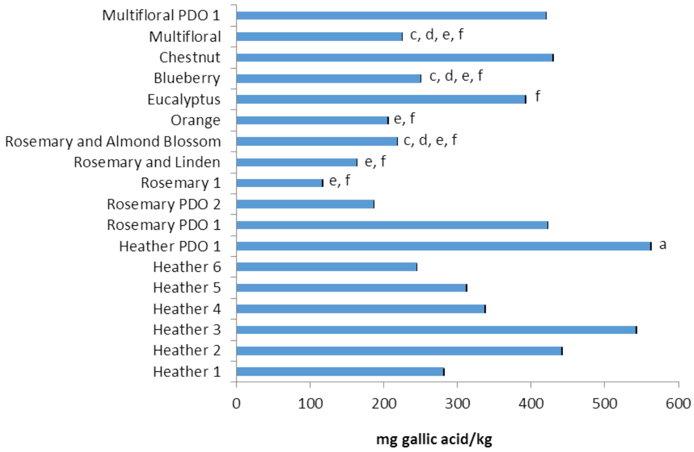


Figure 1. Total phenolic content of the honey samples evaluated.

Different letters represent significant differences (p<0.05) obtained by Scheffe's test.

According to Figure 1, heather honey samples have, in general, the highest contents in phenolic compounds. The sample of heather honey with PDO designation has the highest content (628.50 mg gallic acid/kg) followed by the samples Heather 3 and Heather 2. Rosemary PDO 1 and Multifloral PDO 1 have also high contents of phenolic compounds (480.65 and 475.27 mg gallic acid/kg, respectively). The samples with the lowest values are Rosemary 1 (179.57 mg gallic acid/kg) followed by Rosemary and Linden and Rosemary PDO 2 (227.96 and 265.59 mg gallic acid/kg, respectively).

From all studied samples, 5 are rosemary honeys. These samples presented a high range of values (from 179.57 to 480.65 mg gallic acid/kg).

In general, samples with more than one floral origin (multifloral honeys) presented lower values than the monofloral ones, with the exception of Multifloral PDO 1 honey sample.

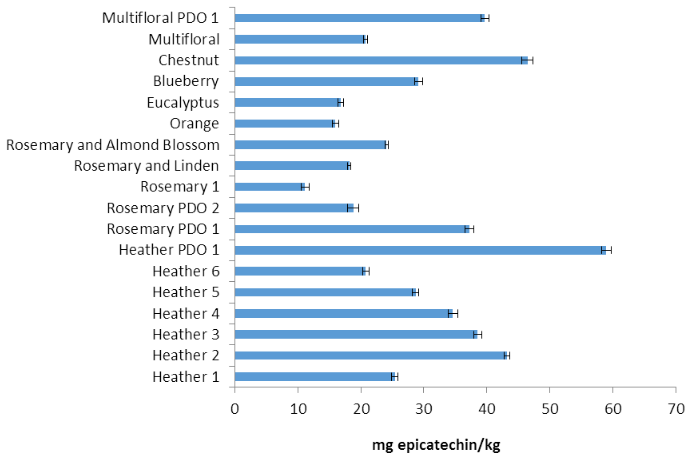


Figure 2. Total flavonoids of honey samples studied.

Different letters represent significant differences (p<0.05) obtained by Scheffe's test.

Regarding the total flavonoids content, it was found that the samples of Heather PDO 1 and Chestnut showed the highest values (58.42 and 47.02 mg epicatechin/kg, respectively), followed by the samples of Heather 2 (45.26 mg epicatechin/kg), Multifloral PDO 1 (38.95 mg epicatechin/kg) and Rosemary PDO 1 (38.68 mg epicatechin/kg). Moreover, samples of Rosemary 1, Orange and Eucalyptus were those with lower total flavonoid content, ranging from 10.18 to 15.88 mg epicatechin/kg.

From the obtained results, in general, darker samples (Heather) showed the highest values for the bioactive compounds analyzed. These results are in agreement with published works about Portuguese honey origins and similar floral origins (Estevinho *et al*., 2012; Ferreira *et al*., 2009).

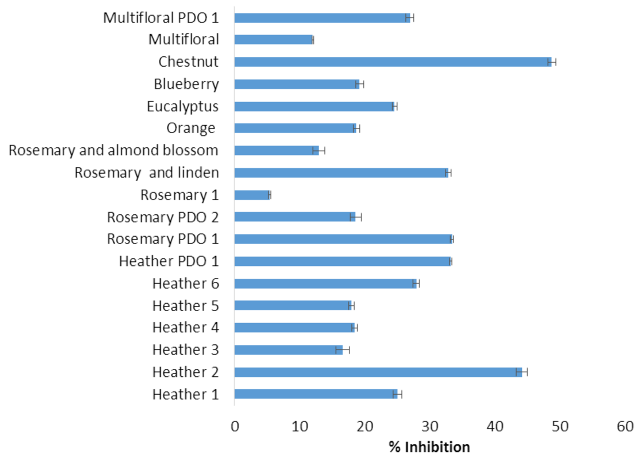


Figure 3. Antioxidant activity of honey samples determined by the DPPH assay and expressed as % inhibition.

Different letters represent significant differences (p<0.05) calculated by Tukey's test.

According to Figure 3, all honey samples analyzed with a concentration of 100 mg/ml, presented inhibition percentages below 50%.

The highest values were achieved for Chestnut and Heather 2 honey samples, closer to 50% inhibition (48.8% and 44.2%, respectively), and so they were considered the samples with higher antioxidant capacity.

On the other hand, one sample of Rosemary honey showed the lowest value (5.4%). Samples of this same floral origin showed values with significant differences, so it was not possible to establish a relationship between the inhibition percentage and the floral origin of the samples.

Concerning to the FRAP analysis (Figure 4) honey samples with higher antioxidant activity were Heather PDO 1 (4086.67 mg ferrous sulphate/kg) and Chestnut (3945.00 mg ferrous sulphate/kg). The sample with the lowest value was Rosemary 1 (509.58 mg ferrous sulphate/kg) followed by Orange (740.83 mg ferrous sulphate/kg) and Rosemary PDO 2 (899.17 mg ferrous sulphate/kg). The statistical analysis did not allow to draw conclusions about a possible relationship between the antioxidant activity of the samples and their floral origin.

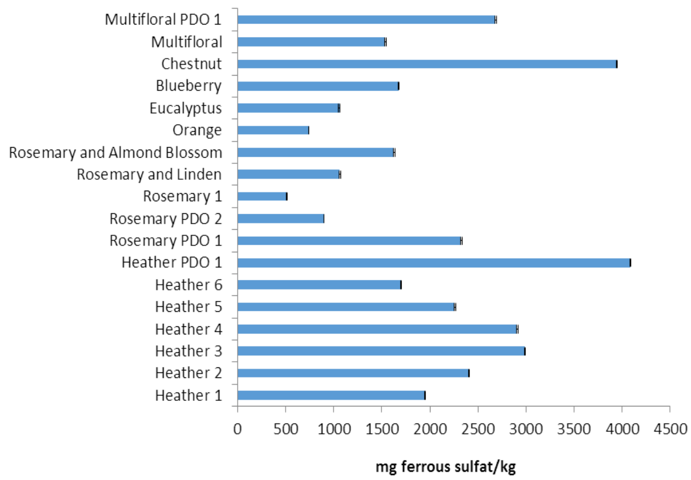


Figure 4. Antioxidant activity of honey samples determined by FRAP method.

Different letters represent significant differences (p<0.05) calculated by Tukey's test

Taking into account the results obtained by both methods and its comparison, in most cases the antioxidant activity of dark honeys was higher than the lighter honeys, according to Bertoncelj *et al.* (2007) and Gheldof and Engeseth (2002). This may be explained, at least in part, by the difference in the phenolic compounds content of the different samples and consequently by its floral origin, as found by Al- Mamary *et al*., (2002).

Conclusion

Among the samples analyzed, it was found that, in general, samples of Heather and Chestnut (dark honeys) showed the highest antioxidant activity and higher levels of total phenols and flavonoids. In contrast, samples of Orchard, Eucalyptus and Rosemary (light honeys) showed the lowest antioxidant capacity related with lower levels of the bioactive compounds determined. Thus, honey color can be considered an indicator of antioxidant activity of this product.

It can also be concluded that the antioxidant activity and the content of total flavonoids and phenolic compounds present in the samples depends on its floral origin and therefore the quality of the final product can be affected by this factor.

Referências

Aljadi, A. M. e Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chemistry, **85**: 513-518.

Al-Mamary, M., Al-Meeri, A. e Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. Nutrition Research. **22**: 1041-1047.

Alves, R. C., Costa, A. S., Jerez, M., Casal, S., Sineiro, J., Nunez, M. J. e Oliveira, B. (2010). Antiradical Activity, Phenolics Profile, and Hydroxymethylfurfural in Espresso Coffee: Influence of Technological Factors. J Agric Food Chem, **11**, 11.

Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chemistry, **63**, 549-562.

Arráez-Román, D., Gómez-Caravaca, A. M., Gómez-Romero, M., Segura-Carretero, A. e Fernández-Gutiérrez, A. (2006). Identification of phenolic compounds in rosemary honey using solid-phase extraction by capillary electrophoresis–electrospray ionization-mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. **41**: 1648-1656.

Azeredo, L. D. C., Azeredo, M. A. A., de Souza, S. R. e Dutra, V. M. L. (2003). Protein contents and physicochemical properties in honey samples of Apis mellifera of different floral origins. Food Chemistry. **80**: 249-254.

Baltrušaitytė, V., Venskutonis, P. R. e Čeksterytė, V. (2007). Radical scavenging activity of different floral origin honey and beebread phenolic extracts. Food Chemistry. **101**: 502-514.

Barroso, M. F. T., Noronha, J. P., Delerue-Matos, C. e Oliveira, M. B. P. P. (2011). Flavored Waters: Influence of Ingredients on Antioxidant Capacity and Terpenoid Profile by HS-SPME/GC-MS. Journal of Agricultural and Food Chemistry. **59:** 5062-5072.

Benzie, I. F. F. e Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. Analytical Biochemistry. **239**: 70-76.

Bertoncelj, J., Doberšek, U., Jamnik, M. e Golob, T. (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chemistry. **105**: 822-828.

Chen, L., Mehta, A., Berenbaum, M., Zangerl, A. R. e Engeseth, N. J. (2000). Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J Agric Food Chem. **48**: 4997-5000.

Cherchi, A., Spanedda, L., Tuberoso, C. e Cabras, P. (1994). Solid-phase extraction and high-performance liquid chromatographic determination of organic acids in honey. Journal of Chromatography A. **669**: 59-64.

DL nº 214/2003 of 18th September, Diário da República Iª Série A.

Estevinho, L. M., Feas, X., Seijas, J. A. e Pilar Vazquez-Tato, M. (2012). Organic honey from Tras-Os-Montes region (Portugal): chemical, palynological, microbiological and bioactive compounds characterization. Food Chem Toxicol, **50**: 258-264.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M. e Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. Food Chemistry. **114**: 1438-1443.

Ferreres, F., Blázquez, M. A., Gil, M. I. e Tomás-Barberán, F. A. (1994). Separation of honey flavonoids by micellar electrokinetic capillary chromatography. Journal of Chromatography A. **669**: 268-274.

Ferreres, F., Andrade, P., Gil, M. e Tomás-Barberán, F. (1996a). Floral nectar phenolics as biochemical markers for the botanical origin of heather honey. Zeitschrift für Lebensmittel-Untersuchung und Forschung. **202**: 40-44.

Ferreres, F., Andrade, P. e Tomás-Barberán, F. A. (1996b). Natural Occurrence of Abscisic Acid in Heather Honey and Floral Nectar. Journal of Agricultural and Food Chemistry. **44**: 2053-2056.

Ferreres, F., Juan, T., Pérez-Arquillué, C., Herrera-Marteache, A., García-Viguera, C. e Tomás-Barberán, F. A. (1998). Evaluation of pollen as a source of kaempferol in rosemary honey. Journal of the Science of Food and Agriculture. **77**: 506-510.

Frankel, S. R., G. E., Berenbaum, M. R. (1998). Antioxidant capacity and correlated characteristics of 14 unifloral honeys. Journal of Apicultural Research, **37**.

Gheldof, N. e Engeseth, N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J Agric Food Chem, **50**: 3050-3055.

Normative Instruction nº 11, of 20th october of 2000, Diário Oficial da União de 23/10/2000, Seccion 1, Page 23.

Küçük, M., Kolaylı, S., Karaoğlu, Ş., Ulusoy, E., Baltacı, C. e Candan, F. (2007). Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chemistry. **100**: 526-534.

Maia, M. (2013). Como melhorar a nossa “performance” para obtermos méis monoflorais? O Apicultor-Revista Trimestral. Acedido em 28 de Novembro 2012, url: http://oapicultor.com/artigos/MEIS_MONOFLORAIS.pdf.

Martos, I., Ferreres, F. e Tomas-Barberan, F. A. (2000a). Identification of flavonoid markers for the botanical origin of Eucalyptus honey. J Agric Food Chem. **48**: 1498-1502.

Martos, I., Ferreres, F., Yao, L., D'Arcy, B., Caffin, N. e Tomas-Barberan, F. A. (2000b). Flavonoids in monospecific eucalyptus honeys from Australia. J Agric Food Chem. **48**: 4744-4748.

Meda, A., Lamien, C. E., Romito, M., Millogo, J. e Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contentes in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry. **91:** 571-577.

Nagai, T., Sakai, M., Inoue, R., Inoue, H. e Suzuki, N. (2001). Antioxidative activities of some commercially honeys, royal jelly, and propolis. Food Chemistry. **75**: 237-240.

Revised codex standard for honey (No. CODEX STAN 12-1981), Rev. 1 (1987). (2001). Codex alimentarius, 11(Rev. 2), 1-8.

Tomás-Barberán, F. A., Martos, I., Ferreres, F., Radovic, B. S. e Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. Journal of the Science of Food and Agriculture. **81**: 485-496.

Honey quality: evolution over storage

Sónia Soares, Diana Nascimento, Anabela S.G. Costa, Rita C. Alves, Antónia Nunes, M. Beatriz P. P. Oliveira *
REQUIMTE, Dep. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal
**email: beatoliv@ff.up.pt*

The quality/authenticity of food is an increasing issue, especially when it concerns to natural food products such as bee products like honey. The major concern of the authorities, consumers, traders and producers is to ensure honey's authenticity, verifying that the product meets all the requirements established by legislation, does not suffer any kind of tampering and keeps all its properties.

The nutritional value of honey as well as its unique flavor, make it an added value product when compared with other sweeteners in the market and, therefore highly susceptible to adulteration (Sivakesava e Irudayaraj, 2002). According to quality requirements from *Codex Alimentarius* (2001) honey for sale should not have added any ingredient, including food additives, or any other compounds that is not part of it composition; should not contain undesirable materials, as well as strange flavor, aroma or odor; must not show signs of fermentation or effervescence and should not be heated or processed, avoiding the change on its composition or impairing its quality (Bogdanov e Martin, 2002).

Honey is composed mainly by carbohydrates (glucose, fructose, maltose, and sucrose), water and minor amounts of organic acids, enzymes, amino acids, pigments, pollen and waxe traces. Some of these compounds are produced by bees and others are derived from plants. Other components can be found in raw honey, such as sugar-tolerant yeasts and dextrose hydrate crystals, beside pollen and wax. In order to destroy yeasts and dissolve dextrose crystals, a controlled heating combined with fine straining or pressure filtration are included in honey processing. The heating application is also useful to reduce honey viscosity, and to facilitate its extraction or filtration. This procedure does not affect honey quality since is very similar to that occur naturally in beehives. However, some honey samples are subjected to high temperatures for liquefaction and pasteurization reasons (Wang and Li, 2011; Anklam, 1998).

Food adulteration is unfortunately a worldwide problem, thus becoming increasingly important a rigorous control of honey's quality to assure its authenticity. Besides being an asset to the producers, it could be a tool to avoid unfair competition, and also answer the high demand of the consumer for a correct information of these products. The authenticity of honey can take into account honey production (addition or removal of components and excessive heating) or honey description (geographical and botanical origin, 'natural', 'organic', 'raw' and 'unheated' honey) (Bogdanov and Martin, 2002).

Honey characterization and its quality evaluation requires the determination of various parameters, such as pollen analysis, color, optical rotation, electrical conductivity, pH and acidity, moisture content, 5-hydroxymethylfurfural (5-HMF) content, sugar composition, proline content, and invertase and diastase activity (Anklam, 1998; Bogdanov *et. al.*, 2004). The 5-HMF content and diastase activity are the main parameters to be taken into account in the assessment of the quality and freshness of honey. In general, the honey with high quality must have a low HMF content and high diastase activity (Tosi *et al.*, 2008).

In the present work the physicochemical parameters: diastase activity (DA), total soluble solids (TSS) content, 5-HMF and color, were determined aiming the evaluation of the effect of storage in the quality of honey. The samples evaluated were heather and orchard honeys from different years (between 2006 and 2011) and local producers. DA, TSS and 5-HMF parameters were determined according Portuguese legislation (Decreto-Lei Nº131/ 85, de 29 de Abril). Color values are presented in the system L*, a*, b*, where L* reflects the brightness of the sample and a* and b* the red-green and yellow-blue coordinates, respectively. Gonzalez-Mirel *et al*. (2005) classified honey into two groups according to their brightness: lighter colored honeys if L*> 50 and dark honeys for L* < 50. While the brightness (L*) varies from 0 to 100, the other two components, a* and b* vary from -100 to +100.

Honey has several compounds in its composition, highlighting small amounts of enzymes, like diastase (α and β-amylase), invertase (α-glucosidase), glucose oxidase, catalase and acid phosphatase. The diastase and invertase enzymes are commonly used to assess the honey freshness, due to their high sensitivity to heat. The diastase activity decreases with honey aging or uncontrolled heating, by structural changes and denaturation (Gonnet, 1965). Although this parameter is closely related to the heat treatment of honey, cannot be used in the evaluation of botanical and/or geographical origin (Anklam, 1998).

According to the Directive 2001/110/EC of 20 December, the minimum value for diastase activity in honey is 8, considering the Gothe scale, with exception of baker's honey. For natural honeys with low content of enzymes (e.g. citrus honey) and 5-HMF content lower than 15 mg/kg, the minimum value of diastase activity is 3 (ºGothe).

The presence of HMF in honey is due to the dehydration of sugars (hexoses) catalyzed by acids. Its content is a honey quality marker, revealing the "aging" of the product. It is generally not present in fresh honey, but its levels show an increasing tendency in honey storage under unsuitable conditions, subjected to excessive heat or adulterated by addition of inverted sugar (Nozal *et al*., 2001).

The heating of honey, in technological terms, is advantageous, since prevents crystallization or fermentation of the product and may even avoid a microbial contamination. Nevertheless an overheating may lead to the production of HMF, contributing to the loss of the honey quality. To avoid the HMF formation, the binomial time/temperature used in the honey processing should be controlled (Tosi *et al*., 2002).

The 5-HMF maximum limit established by Directive 2001/110/EC of 20 December is 40 mg/kg, exception made for honeys from tropical regions and mixtures. In these cases the maximum allowed is 80 mg/kg.

Material and Methods

In this study, 8 honey samples produced by *Apis mellifera* bees in different years, from two different floral origins and two geographical origins (Table 1) were collected from local producers.

Table 1. Description of honey samples analyzed.

Sample	botanical origin	geographical origin	Year of production
M1	Heather	Viseu	2006
M2	Heather	Viseu	2007
M3	Heather	Viseu	2008
M4	Heather	Viseu	2009
M5	Heather	Viseu	2010
M6	Orchard	Gondomar	2009
M7	Orchard	Gondomar	2010
M8	Orchard	Gondomar	2011

Color, total soluble solids (TSS), 5-HMF content and diastase activity were evaluated for all samples and performed in triplicate.

For color analysis a colorimeter CR-400 Chroma Meter (Minolta Konic) was used, previously calibrated with a white standard (L* = 97.71, a* = -0.01, b* = 1.56; system CIELab). The TSS content was determined with a digital refractometer HI 96801, with automatic temperature compensation and according to NP EN 12143:1999. The results are expressed in ˚Brix.

The 5-HMF quantification was performed by the external standard method. For chromatographic analysis was used an HPLC system (Jasco, Japan) equipped with an autosampler (AS-2057 PLUS), a pump (PU-2089 Plus) and a diode detector (MD-2018 plus). The chromatographic separation was performed on a reverse phase column Tracer Excel ODS-A (5 µm; 250 x 4 mm) of Teknokroma (Spain) at 25 °C with isocratic elution (water/acetonitrile, 80:20) with a flow rate of 1 ml/min. The volume injected was 20 µl, the detection was performed at 284 nm and the chromatograms analysed with the Borwin-PDA Controller (JMBS, France) software. The results were expressed as mg/kg honey.

The diastase activity was determined according to the AOAC method 958.09 (AOAC, 2010). The absorbance was read at 660 nm on a spectrophotometer "Perkin-Elmer" (Norwalk, USA). The diastasic index (DI) was calculated by the formula: id = 300/tx (where tx is the total time required, in minutes, for the sample absorbance reaches the value of 0.235). The results were expressed in °Gothe. These results correspond to the amount of enzyme which hydrolyses 1% of starch contained in 1 g of honey in 1 hour.

Results

Table 2 and Figures 1 and 2 show the results of the analysed performed. The values obtained for TSS are fairly consistent, with a mean value of 82% (Table 2). This parameter is related with the total sugars content.

Table 2. Total soluble solids (TSS) content and color values for the honey samples (mean ± standard deviation).

Sample	Total soluble solids (°Brix)	L*	a*	b*
M1	81.4 ± 0.07	33.94 ± 0.13	1.65 ± 0.04	0.97 ± 0.04
M2	81.0 ± 0.00	33.82 ± 0.27	1.61 ± 0.03	0.75 ± 0.03
M3	81.9 ± 0.00	35.80 ± 0.42	3.33 ± 0.10	1.63 ± 0.07
M4	80.8 ± 0.00	35.93 ± 0.05	5.61 ± 0.03	3.41 ± 0.01
M5	83.2 ± 0.00	36.35 ± 0.03	5.36 ± 0.04	4.35 ± 0.03
M6	83.0 ± 0.00	40.49 ± 0.05	4.93 ± 0.05	10.71 ± 0.07
M7	82.6 ± 0.00	35.37 ± 0.13	4.87 ± 0.06	3.03 ± 0.02
M8	82.0 ± 0.00	38.03 ± 0.01	5.72 ± 0.03	7.17 ± 0.03

Honey color is an important quality parameter to the consumers, since it is the first attribute to be observed in the acquisition of the product. Table 2 shows the attributes related to color (L* regarding the light, and a* and b* the chromaticity coordinates). According to Estevinho *et al.* (2012) consumers in Portugal and central Europe prefer dark honeys, as heather honey. In what concerns to color variation, however slight, it seems to be higher in orchard honey than in heather honey samples.

By the analysis of the diastase activity results (Figure 1) most of samples had values below the minimum established by legislation (8 for a 5-HMF content lower than 40 mg/kg). The behavior of the diastase enzyme is related to the overheating or adulteration of honey, due to their sensitivity to heat. It can also be considered a measure of freshness.

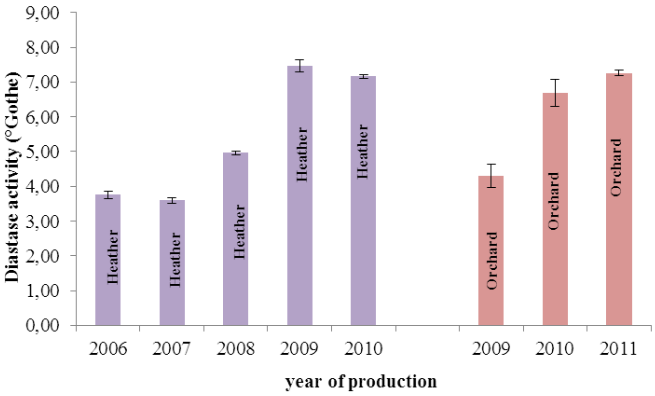


Figure 1. Diastase activity values of the studied honey samples.

The levels of 5-HMF ranged from 0.1 to 4 mg/kg, being heather honey from Viseu produced in 2006 and 2008 the richest samples in this component. The values found are consistent with the age of the honey samples, since it is known that the content of this compound increases with aging.

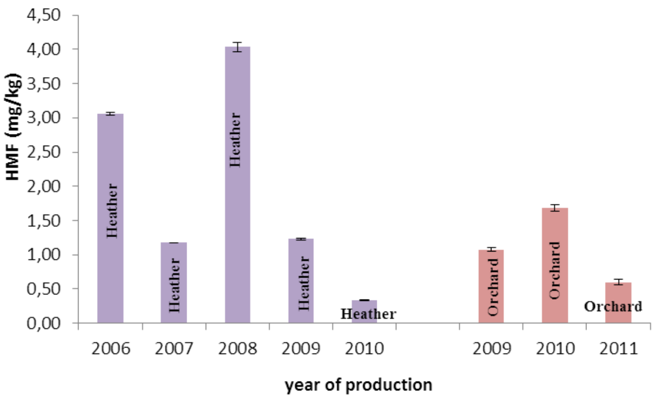


Figure 2. HMF concentration of honey samples studied.

Conclusion

According to the results of this study, it is possible to infer that, in general, honey is a food product that maintains its physical and chemical properties beyond the expiration date. It may therefore continue to be used as a natural sweetener, however to enjoy the enzymes action naturally present in this product (diastase, catalase, acid phosphatase, invertase) should be used a recent honey, since the enzyme activity decreases with time.

References

Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chemistry, 63: 549-562.

AOAC, (2010). Official Methods of Analysis. Association of Official Analytical Chemists. Arlington, USA, 44: 36.

Bogdanov, S. e Martin, P. (2002). Honey authenticity: a review. Mitt. Lebensm. Hyg, 93: 232-254.

Bogdanov, S. Ruoff, K. E Oddo, L. P. (2004). Physico-chemical methods for the characterisation of unifloral honeys: a review. Apidologie, 35: 4-17.

Codex Alimentarius (2001), Revised Codex Standard for Honey.

Decreto de lei nº 131/ 85 de 29 de Abril, Diário da República IªSérie.

Diretiva 2001/110/CE do conselho de 20 de Dezembro de 2001 relativa ao mel. Jornal Oficial das Comunidades Europeias, 10: 47-52.

Estevinho, L. M., Feás, X., Seijas, J. A. e Pilar Vázquez-Tato, M. (2012). Organic honey from Trás-Os-Montes region (Portugal): Chemical, palynological, microbiological and bioactive compounds characterization. Food and Chemical Toxicology, 50: 258-264.

Gonnet, M. (1965). Les modifications de la composition chimique des miels au cours de la conservation Ann. Abeille, 8: 129-146.

Gonzalez-Miret M.L., Terrab A., Hernanz D., Fernandez-Recamales M.A., Heredia F.J. (2005). Multivariate correlation between color and mineral composition of honeys and by their botanical origin, Journal of agricultural and food chemistry, 5: 2574-80.

Nozal, M. J., Bernal, J. L., Toribio, L., Jiménez, J. J. e Martín, M. T. (2001). High-performance liquid chromatographic determination of methyl anthranilate, hydroxymethylfurfural and related compounds in honey. Journal of Chromatography A, 917: 95-103.

NP EN 12141 (1999). Sumos de frutos e de produtos hortofrutícolas. Determinação do teor de sólidos solúveis. Método refractométrico. Instituto Português da Qualidade, Lisboa.

Sivakesava, S. e Irudayaraj, J. (2002). Classification of simple and complex sugar adulterants in honey by mid-infrared spectroscopy. International Journal of Food Science & Technology, 37: 351-360.

Tosi, E., Ciappini, M., Ré, E. e Lucero, H. (2002). Honey thermal treatment effects on hydroxymethylfurfural content. Food Chemistry, 77: 71-74.

Tosi, E., Martinet, R., Ortega, M., Lucero, H. e Ré, E. (2008). Honey diastase activity modified by heating. Food Chemistry, 106: 883-887.

Wang, J. e Li, Q. X. (2011). Chemical composition, characterization, and differentiation of honey botanical and geographical origins. Advances in Food and Nutrition Research, 62: 89-137.

First case of infant botulism in Portugal

Margarida Saraiva, Isabel Campos Cunha, Conceição Costa Bonito, Cláudia Pena, Maria Manuel Toscano, Teresa Teixeira Lopes, Isabel Sousa, Maria Antónia Calhau

Abstract

A clinical case of infant botulism was detected in Portugal. This rare occurrence was detected in a child provided from an immigrant family from Eastern Europe. Apart from breast milk, the parents also gave him honey and chamomile tea. *Clostridium botulinum* type B was identified after bacterial isolation in child faeces, honey and chamomile herbs samples. The Botulism neurotoxin (BoNT) B was detected in the faeces according to CDC Atlanta specific procedures.

© 2012 Elsevier Ltd. All rights reserved.

Keywords

Infant botulism; Honey; Chamomile; Toxin; *Clostridium botulinum*

Full text published on Food Control. 2012 Jul;26(1):79–80. Doi:10.1016/j.foodcont.2012.01.010. Available online: http://www.sciencedirect.com/science/article/pii/S0956713512000114

The honey in the National Sampling Plan and Rapid Alert System (RASFF)

Sónia Ferreira, Paulo Fernandes
Food Risks Unit / ASAE

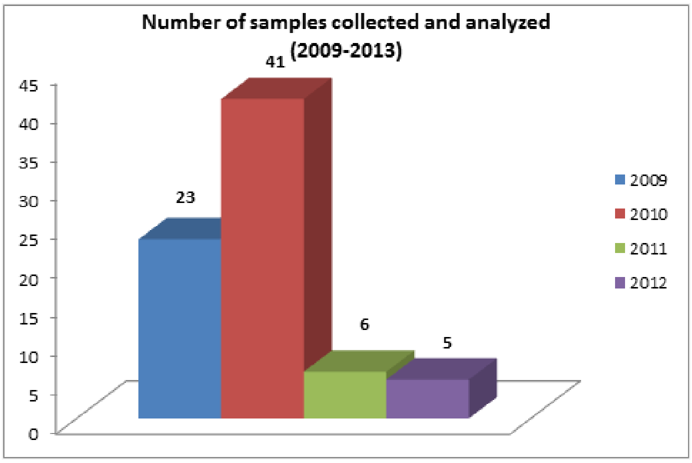
Honey consumption in Portugal

According to Portuguese Food Balance (BAP, 2009) INE, the annual consumption of honey in Portugal is estimated on 0.7 kg by person.

National Sampling Plan

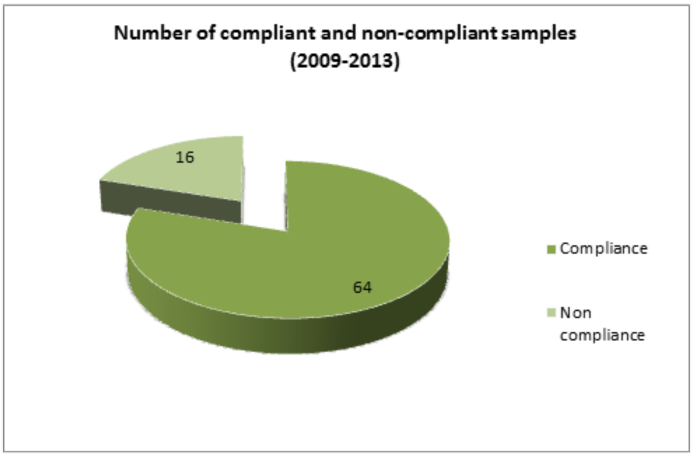
Admitted the importance of the activity of bees, whether bees as pollinators of crops or as producers of honey, with a strong cultural component in Europe and Portugal, it so justified the monitoring of honey under the National Sampling Plan (PNCA).

In this context, in the years 2009 to 2013, 80 honey samples were collected according to the following distribution:



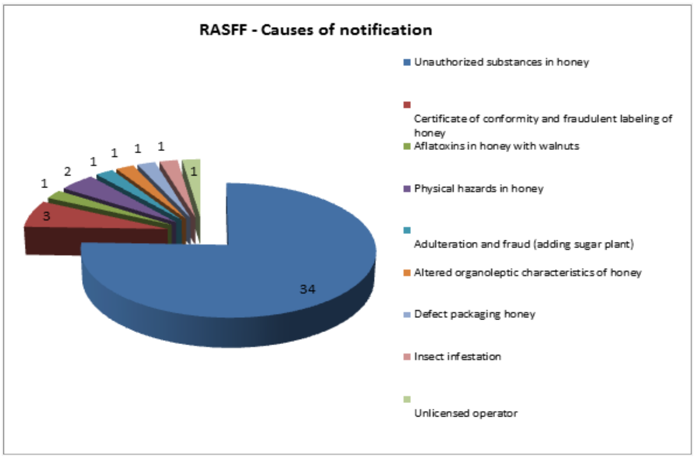
In all samples were researched the presence of hydroxymethylfurfural . The hydroxymethylfurfural (HMF) results from the transformation of fructose and glucose in the honey, which naturally occurs during storage, whenever occurred when honey is subjected to heat and is therefore used as an indicator of honey deterioration and loss of their nutritional value. Honey with HMF above the limit set, in spite of no risk to human health shows a lack of quality requirements.

16 non-compliances were detected about this.



Rapid Alert System

In the same period (2009-2013) 45 alert notifications were issued, being 34 related to the detection of unauthorized substances in honey, including antibiotic residues.



There is made no notice of the presence of HMF on RASFF, because this substance is an indicator of quality rather than safety.

It is important to observe that the main notification cause of the RASFF (presence of unauthorized substances) is not determined in PNCA, since this control is the responsibility of the General Directorate of Food and Veterinary (DGAV), under the National Residue Control Plan (NRCP).

Bibliography

Instituto Nacional de Estatística; Balança Alimentar Portuguesa, INE, 2009

RASFF portal, disponível em: https://webgate.ec.europa.eu/rasff-window/portal/

The National Sampling Plan (PNCA) is an official control plan coordinated and implemented by ASAE, which purpose is to verify that the existing food in market does not endanger human safety and health. The importance of this objective is based on the analysis of the compliance of foodstuffs, accordant with is stipulated in the Community and National legislation, microbiologic, chemical, physical and technological parameters levels, and also in relation to the labeling, presentation and marketing. Obtained laboratory results, besides to allowing (in the strict sense) the type of analysis indicated, provide (after treated), a whole set of information and experience to carry for the design of future monitoring activities, especially with the priorities to establish (an aspect that is contemplated in the ASAE strategy).

Food Safety in schools

Maria Manuel Mendes, *Head of Division of Food Risks Unit/ASAE;*
Maria Manuela Sol, *Head of Microbiology Laboratory/ASAE.*

One of the ASAE competencies, within the definition of the strategies of theRisk Communication on food safety, is the diffusion of useful information to the public on food safety. In this context and integrating the plan of promotion of health education, the Food Risks and Laboratories Department has developed two projects: the "*Safe Food*" project and the "*Clean hands*" project, for children in primary and secondary schools.

The children are moved to have small hygiene gestures such as hand washing, as well as to know some basic rules on food safety, like the importance of temperature in food preservation, for preventing several diseases.

This initiative, which began in October is being developed in partnership with school groups, and has already reached more than two thousand and five hundred students across the country in several schools, and we hope to reach many more.

The success we have had in schools and the great support and interest shown by the students, allows us, by now, to know that this initiative is a complete success . Thus, because the students and teachers reception to is very good , there are already others proposals that will be continuing this pioneer and innovator ASAE's project.

The "*Safe Food*" project addresses each session related matters:

- Basic hygiene (the importance of washing hands);
- How to avoid diseases always aiming the foodstuff preservation;
- Importance of the temperature in food preservation;
- How to make a proper choice of food: the reading of food labels more carefully , more knowledge about additives;
- Correct way to make a safe distribution of food inside your refrigerator.

For the "*Clean Hands* project - The Lab Goes to School", each session is organized in three stages:

- 1st Stage: "The Laboratory" goes to school and students put cleaned and disinfected hands and unwashed hands in Petri dishes with culture media;
- 2nd Stage: The Petri dishes will be incubated in the ASAE's Laboratory of Microbiology incubator;
- 3rd Stage: "The Laboratory" is back to school and shows the result of experience;
- This project intends, by experimental observation, to internalize the importance of always to have "*Clean Hands*".

Specifications:
Riscos e Alimentos, nr. 6
december 2013

Property:
Authority for Food and Economic Safety
(ASAE)

Coordinated, Edition and Revision by:
(DRAL) /UNO

Distribution:
DRAL / UNO

Periodicity :
Semiannual