Putative Anticataract Properties of Honey Studied by the Action of Flavonoids on a Lens Culture Model

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Stingless bee (Meliponini) honey is a bioresource used to treat cataracts in traditional medicine. The anti-cataract activity of twenty flavonoids was explored in an osmotic cataract model, to find a probable link between the putative anti-cataract properties of stingless bee honey eyedrops and their flavonoids. Osmotic cataracts were induced in ovine lenses to produce a model to test anti-cataract drugs in cultured lenses by digital image analysis. Digital images were taken every 4 hr to monitor progressive opacification by measurements of grey level. In 24 hr. the opacification was stable. Osmotic cataracts were induced by incubating ovine lenses in 45% hypotonic HBS for 24 hr to test the anticataract action of twenty synthetic flavonoids at a concentration of 10^{-5} M. Luteolin tetramethyl ether, luteolin 4′-glucoside, luteolin 3′-7-diglucoside and orientin, significantly inhibited cataracts induced in ovine lenses incubated in 45% hypotonic HBS for 24 hr. Different degrees of opacification were produced by hypotonic stress in ovine lenses. The significant inhibition of cataracts caused by four derivatives of luteolin *in vitro* may be considered as a preliminary evidence for the putative anticataract properties of stingless bee honeys.

Key words —— cataract, honey, flavonoid, luteolin, osmotic, digital images

INTRODUCTION

Ocular cataract is lens opacification. At least 50 million people suffer from cataract-related visual impairments, with 17 million being severely disabled. In the U.K., cataract is one of the four main causes of blindness. Half of the blindness in the world is due to cataract and this represent some 13 million people.[1)] Surgery to implant intraocular lenses is the actual treatment of cataracts currently accepted.[2, 3)] However, the prescription of anticataract agents of different nature is a common practice although their action on cataracts have not been properly supported by prospective clinical trials.[4)] Following the sorbitol theory of cataractogenesis,[5)] aldose reductase inhibitors (ARI) were studied as promising agents for the medical treatment of cataracts, and their action to inhibit the onset of cataracts has been tested both *in vitro* and *in vivo*.[6, 7)] Some flavonoids have been reported as anticataract agents in several studies *in vitro* because of their action as ARI.[8–10)]

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The use of stingless bee (Hymenoptera; Apidae; Meliponinae) honey as eyedrops to cure cataracts is a folk practice in tropical countries of America[11)] dating back to the Mayas,[12)] but there is no scientific evidence available on that ancient remedy. In a recent review of medicinal properties attributed to these non-*Apis* honey type, the treatment of pterygium is another benefit suggested for the topical application in the eyes.[13)] Plant extracts are also added to stingless bee honey sold for eye treatment.[14)] Flavonoids are found in honey as residual secondary plant metabolites, and have been studied for their potential use as botanical and geographical markers,[15–20)] and also to explain the antibacterial properties of honey.[21, 22)] However, flavonoids have not been previously explored in connection with the putative anticataract attributes of honey. In a recent study, the flavonoid diversity of *Apis mellifera* honeys from Venezuela was similar to *Melipona* spp. but lower than the diversity reported for temperate honeys,[23)] with frequent luteolin derivatives in the phenolic fraction.[24)]

An initial approach to validate the reputed anticataract properties of honey is a monofactorial study considering the effects of flavonoids present in honey on a cataract model *in vitro*, prior to any *in vivo* study. In the present work, a method

was explored to produce *in vitro* controlled osmotic cataracts incubating ovine lenses in hypotonic culture media. This method was used to test the anticataract action of twenty synthetic flavonoids at a concentration of 10^{-5} M.

MATERIALS AND METHODS

Culture Media —— Hepes buffered solution (HBS) was the control solution used for lens culture. The HBS was prepared by dissolving 7.305 g NaCl (125 mM), 0.328 g KCl (4.4 mM), 0.840 g $NaHC0_3$ (10 mM), 2.383 g Hepes (10 mM), 0.900 g glucose (5 mM), 6.864 g sucrose (20 mM), 2 ml 1 M $CaCl_2$ (2 mM) and 100 µl 5 M $MgCl_2$ (0.5 mM), to 800 ml of distilled water. The pH was adjusted to 7.4 with 1N HCl and 1N NaOH solutions. The final volume was brought up to 1 l with distilled water and the solution was sterilised with a 0.22 µm bottle top filter (Falcon 7105, from Becton Dickinson, Oxford, UK). The osmolality of the HBS was measured using an automatic cryoscopic osmometer (Osmomat 030, Gonotec, Berlin, Germany) and adjusted to 310 ± 10 mosmol kg^{-1} with distilled water. This solution was freshly prepared at the beginning of each experiment, and kept at 4°C. To prevent infection, 2 ml antibiotics/100 ml HBS were added just before use. The stock antibiotic solution was made up with penicillin (5000 IU/ml) and streptomycin (5000 µg/ml).

Eyes —— Enucleated eyes from *Ovis aries* were obtained in a local abattoir within 1–2 hr post mortem and stored at 4°C up to 2 hr before use.

Dissection of Eyes and Lens Preparation —— The ovine eyes were opened by a scalpel incision at the corneal limbus. The cornea was removed cutting with scissors around its junction with the sclera. The exposed lens was kept moist with HBS + P/S. The iris was held with forceps and gradually removed. Finally the suspensory ligaments were cut with fine curved scissors, the lens was removed with a loop and stored in a 50 ml sterile container with control culture medium.

Lens Incubation —— Three lenses were distributed at random for each treatment after prewarming to 37°C in a humidified incubator (Gallenkampf, from Fisher Scientific, Loughborough, UK) for 15 hr prior to the experiment, to permit equilibration. Individual lenses were immersed in 5 ml of prewarmed HBS into a sterile glass tube (2.5 cm diameter). These experimental units were incubated in a plastic box at 37°C to prevent evaporation.

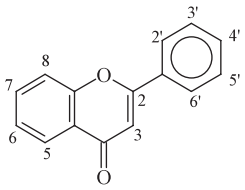
Osmotic Cataract Model —— HBS solutions were modified by the addition of distilled water to make a series of hypotonic solutions: 30%, 35%, 40%, 45% and 50% hypotonic. Lenses were incubated as described above at 37°C for 24 hr. These hypotonic solutions were found to induce opacification (cataract) of increasing severity. The degree of opcification was measured as detailed below. The opacification caused by both 45% and 50% hypotonic solutions was similar therefore 45% hypotonic solution was chosen to test the anti-cataract properties of flavonoids.

Flavonoid Treatments —— Apigenin (Sigma, Poole, Dorset, UK), acacetin, acacetin 7-O-α-rutinoside, apigenin 7-O-α-glucoside, avicularin, homorientin, hyperoside, isoquercitrin, kaempferol, kaempferol 3-O-α-robinoside-7-O-α-rhamnoside, luteolin, luteolin 7-O-α-glucoside, luteolin tetramethylether, luteolin 4′-O-α-glucoside, luteolin 3′-7-O-α-diglucoside, orientin, peltatoside, quercetin, quercetin 3,7,3′,4′-tetramethylether and quercetin 3-O-β-D-glucopyranosyl-6′-acetate (Extrasynthese) were solved in 50 µl propyleneglycol to prepare 100 ml HBS solutions 10^{-5} M of each flavonoid. The flavonoid structures are indicated in Table 1.

Digital Images —— Digital photographs of polar views of the lenses with a black background were periodically taken every 4 hr with a charged coupled device camera (CCD EDC-1000HR, Electrim Corporation, Princeton, New Jersey, USA), during 24 hr for the osmotic cataract model. For the flavonoid test, only initial (0 hr) and final (24 hr) images were saved. The conditions of the camera were set up with a black card as a standard for control.

Measurements of Grey Level —— The digital images of polar views were analysed with Quantimet Q500MC (Leica, Milton Keynes, UK) software to measure the mean grey level of the pixels contained in the image, a quantity referred to as density of white. The scale of grey level varies from 0 (black) to 255 (white). The measurements were done following the routine program Quantimet Image Processing Software (QUIPS, Leica) designed to locate the lens, to detect the threshold grey level in the border of the lens, to close it with a line when necessary, to fill holes, to subtract the border, to fill holes again, to reject areas outside the lens and finally to measure the average grey level of the pixels in the isolated two-dimensional image of the lens. The simplest cataract index to quantify different degrees

Table 1. Structures of Flavonoids Tested in Cataract Model



Flavonoids	Abbreviation	substituents						
		R_3	R_5	R_6	R_7	R_8	$R_{3'}$	$R_{4'}$
Acacetin	aca	H	OH	H	OH	H	H	OCH_3
Acacetin 7-O-α-rutinoside	aca7R	H	OH	H	Orut	H	H	OCH_3
Apigenin	api	H	OH	H	OH	H	OH	H
Apigenin 7-O-α-glucoside	api7G	H	OH	H	Oglu	H	OH	H
Kaempferol	kae	OH	OH	H	OH	H	H	OH
Kaempferol 3-O-α-robinoside-7-O-α-rhamnoside	kae3R7H	Orob	Orha	H	OH	H	H	OH
Luteolin	lut	H	OH	H	OH	H	OH	OH
Luteolin tetramethylether	lutMe4	H	OCH_3	H	OCH_3	H	OCH_3	OCH_3
Luteolin 6-C-glucoside (homorientin)	lut6G	H	OH	Cglu	OH	H	OH	OH
Luteolin 8-C-glucoside (orientin)	lut8G	H	OH	H	OH	Cglu	OH	OH
Luteolin 3′,7-O-α-diglucoside	lut3′G7G	H	OH	H	Oglu	H	Oglu	OH
Luteolin 4′-O-α-glucoside	lut4′G	H	OH	H	OH	H	OH	O-glu
Luteolin 7-O-α-glucoside	lut7G	H	OH	H	Oglu	H	OH	OH
Quercetin	que	OH	OH	H	OH	H	OH	OH
Quercetin-3-O-α-arabinoside (avicularin)	que3A	Oara	OH	H	OH	H	OH	OH
Quercetin-3-O-α-arabinoglucoside (peltatoside)	que3AG	Oara/glu	OH	H	OH	H	OH	OH
Quercetin-3-O-α-galactoside (hyperoside)	que3L	Ogal	OH	H	OH	H	OH	OH
Quercetin 3-O-α-glucoside (isoquercitrin)	que3G	Oglu	OH	H	OH	H	OH	OH
Quercetin 3-O-β-D-glucopyranosyl-6″-acetate	que3GAc	Oglu/Ac	OH	H	OH	H	OH	OH
Quercetin 3,7,3′,4′-tetramethylether	queMe4	OCH_3	OH	H	OCH_3	H	OCH_3	OCH_3

of opacification was considering the value of grey level as a measurement of lens transparency. Due to the black background, control lenses are darker than opacified lenses, and therefore have lower values of grey level than opacified lenses with cataracts causing a higher density of white.

RESULTS

Cataracts were successfully produced in all the hypotonic (30–50%) culture media. The progressive development of these osmotic cataracts was measured every four hours for 24 hr and is presented in Table 2.

The lenses incubated in 30% and 35% hypotonic HBS, reached their maximum opacification in eight hours. At higher hypotonicity, the lenses required 16 hr to maximize their opacification. In 24 hr. the opacification was stable. Osmotic cataracts obtained with 45% and 50% hypotonic HBS overlapped in the final measurements, therefore the HBS 45% hypotonic was selected to produce cataracts in the present model.

The opacification of the lenses exposed to a 45% hypotonic HBS and flavonoids (10^{-5} M) for 24 hr, is presented in Table 2. All the treatments were significantly different (ANOVA one-way, $\alpha = 0.05$) from the isotonic control because cataracts developed in the presence of any flavonoid, but with different severity. Only four of the flavonoids (luteolin tetra-methyl ether, luteolin 4′-glucosyde, luteolin 3′-7-diglucosyde and orientin) significantly reduced the opacification caused by the 45% hypotonic culture media, with average grey levels in the range 116.00–121.60 detected by the method as significantly different [analysis of variance (ANOVA) one-way, $\alpha = 0.05$] from other tested flavonoids.

Both anterior and posterior cortical cataracts developed simultaneously, increasing their severity

Table 2. Effect of Flavonoids 10^{-5} M in Cataract Model

Treatments		Grey scale[a)]	Treatments		Grey scale[a)]
Isotonic HBS	control	90.87 ± 0.39** (90.10 – 91.40) [0.75]	Acacetin		142.95 ± 5.15 (137.80 – 148.10) [5.09]
Luteolin 4′-O-α-glucoside		116.00 ± 1.57* (113.70 – 119.00) [2.34]	Apigenin 7-O-α-glucoside		144.90 ± 5.10 (139.80 – 150.00) [4.98]
Luteolin 3′-7-O-α-diglucoside		117.20 ± 1.20* (116.00 – 119.60) [1.77]	Quercetin 3,7,3′,4′-tetramethylether		145.53 ± 2.13 (142.70 – 149.70) [2.54]
Luteolin tetramethylether		120.13 ± 1.87* (116.5 – 122.70) [2.69]	Hyperoside		145.67 ± 2.34 (141.00 – 148.40) [2.79]
Orientin		121.60 ± 7.92* (107.20 – 134.50) [11.28]	45% hypotonic HBS	osmotic cataract	145.83 ± 2.84 (142.80 – 151.50) [3.37]
Isoquercitrin		136.13 ± 3.20 (130.00 – 140.80) [4.08]	Avicularin		146.17 ± 2.59 (142.60 – 151.20) [3.06]
Kaempferol		139.45 ± 5.35 (134.10 – 144.80) [5.51]	Luteolin 7-O-α-glucoside		148.05 ± 1.85 (146.20 – 149.90) [1.77]
Luteolin		140.15 ± 9.95 (130.20 – 150.10) [10.04]	Acacetin 7-O-α-rutinoside		148.55 ± 3.35 (145.20 – 151.90) [3.19]
Quercetin 3-O-β-D-glucopyranosyl-6′-acetate		141.33 ± 4.96 (134.90 – 151.10) [6.09]	Peltatoside		151.33 ± 7.05 (138.90 – 163.30) [8.07]
Kaempferol 3-O-α-robinoside-7-O-α-rhamnoside		141.45 ± 2.65 (138.80 – 144.10) [2.65]	Apigenin		152.90 ± 1.90 (151.00 – 154.80) [1.76]
Homorientin		142.40 ± 6.35 (134.20 – 154.90) [7.73]	Quercetin		154.73 ± 9.42 (143.20 – 173.40) [10.54]

a) Values are grey scale mean ± SEM, (ranges) and [cv]. The grey scale varies between 0 (black) and 255 (white) ** different from other treatments ($p < 0.050$) * different from isotonic HBS and 45% hypotonic HBS ($p < 0.050$) after *t*-student test.

from the periphery to the centre of the lens, no nuclear cataracts were observed. Variations of cataract morphology according to cataract severity will be discussed elsewhere. Lenses treated with luteolin tetra-methyl-ether generated a characteristic pattern of opacification in their equatorial view; complete anterior and partial posterior cataracts occurred with an even ring-like band extending under the equator, leaving the posterior pole of the lens without opacification.

DISCUSSION

Osmotic cataracts produced by incubating ovine lenses in hypotonic culture media are simple in their nature of involving a water imbalance. The density of white increases with the hydration of the cells because their swelling scatters the light and alters the optical property of clarity in a normal lens.[25] The loss of transparency was visible after 4 hr of hypotonic stress and developed to a maximum of 120 grey level units from the basal value of 90 grey level units of the control in isotonic HBS. The osmotic stress generated by 45% hypotonic HBS was the minimum required to reach the maximum opacification detected with the method. Less severe treatments are more likely to recover in the presence of anticataract agents. Experimental recovery or cataract reabsorption has been observed in lenses incubated in 30% hypotonic HBS and returned to isotonic culture medium (unpublished data). The

progression of experimental cataracts from the periphery to the polar regions of the lens will require a further different form of assessment to compare the equatorial band with the central polar area as a function of time.

Flavonoids are most commonly known for their antioxidant activity. However, interest in the context of the eye stems from their ability to inhibit aldose reductase, the enzyme implicated in the aetiology of cataract in diabetic and galactosemic rats. Over forty flavonoids were found to be inhibitors of aldose reductase, the two most potent being quercitrin and quercitrin-2″-acetate.[8] Subsequent *in vivo* tests, demonstrated that quercitrin decreased the accumulation of sorbitol and fructose in the lens, and delayed cataract formation in diabetic degus (small rodents). The aglycone quercetrin delayed lens damage in galactose fed rats.[26] Neither isoquercitrin nor quercetin reduced the opacification in our osmotic cataract model, indeed, there is some evidence that quercetin exacerbated the opacification. In this context it is interesting that quercetin has been shown to inhibit the Na^+/K^+-ATPase[27] and it has long been known that inhibition of the Na^+/K^+-ATPase causes cataract.[28] However, substituted derivatives of luteolin all caused a significant reduction in the degree of opacification after 24 hr.

In a different approach to assess the anticataract activity of commercial natural products by monitoring the inhibition of sorbitol metabolism using ^{13}C-nuclear magnetic resonance, methylated flavonoids were stronger inhibitors than the non methylated.[29] In the present work, both methyl and glucosyl substituents increased the percentage of cataract inhibition by the aglycone luteolin, except the 7-glucosyl, but it was also observed that only the methylated luteolin caused a distinctive pattern of complete cataract inhibition in the posterior pole of the lens. The explanation of this final observation requires further investigation.

Flavonoids have been shown to possess many properties; they inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca^{+2}-ATPase, lipoxygenase, cycloxygenase. They also have a regulatory role on different hormones like estrogens, androgens and thyroid hormone.[30] Different flavonoids exhibit different effects and specificities. Flavonoid aglycones have different properties to their glycosylated counterparts, at least in terms of their effect on phosphodiesterases.[31] In our study there was no correspondence between the flavonoids found by Varma and Kinoshita[8] to have aldose reductase inhibitory properties and the flavonoids preventing opacification in our model. They found di-, tri-, tetra- and penta-hydroxy flavonoids with inhibitory properties, both with and without glycosyl substitutions. We, on the other hand found that it was glycosylated or substituted tetrahydroxy-flavonoids that had the most pronounced effect. Di-, tri- and penta-hydroxy flavonoids were ineffective. We can infer therefore that the action is probably not via inhibition of aldose reductase.

Luteolin is a frequent flavonoid in the phenolic profile of *Melipona* spp. from Venezuela;[23,24] therefore, the fact that in the present flavonoid screening, luteolin derivatives were more likely to be involved in a mechanism of cataract inhibition provides an interesting connection with the putative anticataract properties of stingless bee honeys. Due to the dual nature of honey, being both natural medicine and food, a complementary area for further investigation is the anticataract effect of dietary flavonoids derived from stingless honey. The wide use of honey for medicinal purposes demands pharmaceutical regulation besides its use as a food sweetener. Stingless bee honeys are not included in the *Codex Alimentarius* because only the sweet product kept in combs by *Apis mellifera* is currently defined as honey.[32] Therefore, the creation of regulations for stingless bee honey are a must to control the quality of eye drops for this traditional anticataract practice to be adopted in modern medicine.

Reviewed aspects on anti-inflammatory action, antioxidant activity, stimulation of cell growth, the release of hydrogen peroxide, and the harmlessness of honey provide the therapeutical rationale for the use of honey as a standard medicine.[33] However, the mechanisms suggested to explain the effectiveness of honey as a medicine need the understanding of its multifactorial composition and multivariate nature causing systemic or localized physiological responses. Honey is a healing fluid with more variation than any medicinal herb because nectar is originated from different plants. In this context, the work done on sensorial evaluation of honey, initiated in France,[34] has provided extensive knowledge on how different is one honey from another with different botanical origin. In 1990 an International Honey Commission, chaired by Dr. Bogdanov, was created to review the official methods of *Apis mellifera* honey analysis. A recent proposal to expand honey standards to all species of bees,

foresee the adoption of analytical methods used to assess and to harmonize chemical composition and botanical origin of *Apis mellifera* honey, but also needs new methods to identify the entomological origin.[35] Admirable contributions to inform apidologists and the public on honey types can be illustrated by ministerial projects like the guide to value honey according to denominations of origin[36] and the special issue of Apidologie (2005) on European unifloral honeys.[37] This year, special attention was also given to the flavonoid content of food, including 2.5 mg/100 g honey.[38]

The domestication of stingless bees to facilitate honey harvesting, will be merited if their anticataract properties are sufficiently demonstrated. Technical solutions for the design of stingless bee hives already exist,[39] but integrated efforts addressed to meliponiculture, such as the Regional Programme on Tropical Beekeeping in Costa Rica,[40] are also necessary for a consistent progress in the quality control of this ancient honey. Stingless bee eyedrops could be applied prior to the consideration of surgical solutions, together with preventive nutritional approaches[41] and protective habits against environmental UV light[42] to delay the onset of cataracts.

This study provides initial results to approach the understanding of an ancient belief on the use of honey to clarify vision. Flavonoids are one of the possible active principles contained in honey. However, due to the chemical diversity of the nature and position of substituents, not all flavonoids have anticataract action. Here, we demonstrated that four structures of the luteolin family caused reduction of opacification in a lens culture model for osmotic cataract.

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REFERENCES

1) Douthwaite, W. A. (1993) Introduction. In *Cataract. Detection, Measurement and Management in Optometric Practice* (Douthwaite, W. A. and Hurst, M. A., Eds.), Butterworth Heinemann, Oxford, pp. 1–3.
2) Harding, J. J. (1991) *Cataract: Biochemistry, Epidemiology and Pharmacology*, Chapman & Hall, London.
3) Weatherill, J. R. (1993) The surgical management of age-related cataract. In *Cataract. Detection, Measurement and Management in Optometric Practice* (Douthwaite, W. A. and Hurst, M. A., Eds.), Butterworth Heinemann, Oxford, pp. 66–77.
4) Young, S. A. (1993) The medical treatment of cataract. In *Cataract. Detection, Measurement and Management in Optometric Practice* (Douthwaite, W. A. and Hurst, M. A., Eds.), Butterworth Heinemann, Oxford, pp. 128–136.
5) van Heyningen, R. (1959) Formation of polyols by the lens of the rat with 'sugar' cataract. *Nature*, **184**, 194–195.
6) Okuda, J., Miwa, I., Inagaki, K., Horie, T. and Nakayama, M. (1982) Inhibition of aldose reductases from rat and bovine lenses by flavonoids. *Biochem. Pharmacol.*, **31**, 3807–3822.
7) De Santis, L. (1986) New horizons in the medical therapy of cataract: aldose reductase inhibitors and other agents. *Pharm. Int.*, **7**, 17–20.
8) Varma, S. D. and Kinoshita, J. H. (1976) Inhibition of lens aldose reductase by flavonoids—their possible role in the prevention of diabetic cataracts. *Biochem. Pharmacol.*, **25**, 2505–2513.
9) Varma, S. D., Mizuno, A. and Kinoshita, J. H. (1977) Diabetic cataracts and flavonoids. *Science*, **195**, 205–206.
10) Kador, P. F. and Kinoshita, J. H. (1984) Diabetic and galactosaemic cataracts. In *CIBA Symposium 106 on Human Cataract Formation* (Nugent, J. and Whelan, J., Eds), Pitman, London, pp. 110–131.
11) Shimizu, M., Ito, T., Terashima, S., Hayashi, T., Arisawa, M., Morita, N., Surokawa, S., Ito, K. and Hashimoto, Y. (1984) Inhibition of lens aldose reductase by flavonoids. *Phytochemistry*, **23**, 1885–1888.
12) Vit, P. (1994) Las abejas criollas sin aguijón (1). *Vida Apícola*, **63**, 34–42.
13) Vit, P., Medina, M. and Enriquez, E. (2004) Quality standards for medicinal uses of Meliponinae honey in Guatemala, Mexico and Venezuela. *Bee World*, **85**, 2–5.
14) Vit, P. (1997) Stingless bee honey and the treatment of cataracts. In *Honey and Healing* (Munn, P. and Jones, R., Eds.), International Bee Research Association, Cardiff, pp. 37–40.
15) Amiot, M. J., Aubert, S., Gonnet, M. and Tacchini, M. (1989) Les composés phénoliques des miels: étude préliminaire sur l'identification et la quantifi-

cation par families. *Apidologie*, **20**, 115–125.
16) Sabatier, S., Amiot, M. J. and Tacchini, M. (1992) Identification of flavonoids in sunflower honey. *J. Food Sci.*, **57**, 773–774, 777.
17) Ferreres, F., García-Viguera, C., Tomás-Lorente, F. and Tomás-Barberán, F. A. (1993) Hesperetin: A marker of the floral origin of Citrus honeys. *J. Sci. Food Agric.*, **61**, 121–123.
18) Tomás-Barberán, F. A., Ferreres, F., García-Viguera, C. and Tomás-Lorente, F. (1993) Flavonoids in honey of different geographical origin. *Z. Lebensm. Unters. Forsch.*, **196**, 38–44.
19) Ferreres, F., Andrade, P. and Tomás-Barberán, F. A. (1994) Flavonoids from Portuguese heather honey. *Z. Lebensm. Unters. Forsch.*, **199**, 32–37.
20) Soler, C., Gil, M., García-Viguera, C. and Tomás-Barberán, F. A. (1995) Flavonoid patterns of French honeys with different floral origin. *Apidologie*, **26**, 53–60.
21) Bogdanov, S. (1984) Characterisation of antibacterial substances in honey. *Lebensm. Wissensch. Technol.*, **17**, 74–76.
22) Bogdanov, S. (1989) Determination of pinocembrin in honey by using HPLC. *J. Apic. Res.*, **28**, 55–57.
23) Vit, P., Soler, C. and Tomás-Barberán, F. A. (1997) Phenolic profiles of Apis mellifera and Melipona spp. honeys from Venezuela. *Z. Lebensm. Unters. Forsch.*, **204**, 43–47.
24) Vit, P. and Tomás-Barberán, F. A. (1998) Flavonoids in Meliponinae honey from Venezuela, related to their botanical, geographical and entomological origin to assess their putative anticataract properties. *Z. Lebensm. Unters Forsch.*, **206**, 288–293.
25) Paterson, C. A. (1986) *Crystalline Lens* (Thomas, D. D. and Jaeger, E. A., Eds.), Biomedical Foundations of Ophthalmology, vol.2, Harper & Row Publishers, pp. 1–26.
26) Beyer-Mears, A. and Farnsworth, P. N. (1979) Diminished sugar cataractogenesis by quercetin. *Exp. Eye Res*., **28**, 709–716.
27) Carpedeno, F., Bortignon, C., Bruni, A. and Santi, R. (1969) Effect of quercetin on membrane—linked activities. *Biochem. Pharmacol.*, **18**, 1495–1500.
28) Harris, J. E. and Gruber, L. (1962) The electrolyte and water balance of the lens. *Exp. Eye Res.*, **1**, 372–386.
29) Chiou, G. C. Y., Stolowich, N. J., Zheng, Y. Q., Shen, Z. F., Zhu, M. and Min, Z. D. (1992) Effects of some natural products on sugar cataract studied with Nuclear Magnetic Resonance Spectroscopy. *J. Ocul. Pharmacol.*, **8**, 115–120.
30) Narayana, K. R., M. S. Reddy, M. S., Chaluvadi, M. R. and Krishna, D. R. (2001) Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. *Indian J. Pharmacol.*, **33**, 2–16.
31) Beretz, A., Anton, R. and Cazanave, J.-P., (1986) The effects of flavonoids on cyclic nucleotide phosphodiesterases. *Prog. Clin. Biol. Res.* **213**, 281–296.
32) Vit, P., Persano Oddo, L., Marano, M. L. and Salas de Mejías, E. (1998) Venezuelan stingless bee honeys characterised by multivariate analysis of compositional factors. *Apidologie*, **29**, 377–389.
33) Molan, P. C. (1999) Why honey is effective as a medicine? I. Its use in modern medicine. *Bee World*, **80**, 80–92.
34) Gonnet, M. and Vache, G. (1986) *Le Goût du Miel*, Editions UNAF, Paris.
35) International Honey Commission (2001) Report of a meeting, Athens 27/28 September 2001, http://www.apis.admin.ch/english/host/pdf/honey/MinutesAthens.pdf (accessed 29.5.2004)
36) Persano Oddo, L. and Piana, M. L. (2001) *Miele e Territorio: Guida alla Valorizzazione del Miele Attraverso le Denominazioni di Origine*, Ministero delle Pollitiche Agricole e Forestali, Istituto Sperimentale per la Zoologia Agraria, Roma.
37) von der Ohe, W. (guest editor) (2005) European Unifloral Honeys. *Apidologie* (Special issue), **35**, s1–s111.
38) US Department of Agriculture (USDA) (2007) *Database for the Flavonoid Content of Selected Foods*, Release 2.1. Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture, USDA, Betsville.
39) Nogueira-Neto, P. (1970) *A Criaçao de Abelhas Indígenas sem Ferrâo*, Editora Chácaras e Quintais, Sâo Paulo.
40) Sommeijer, M. (1996) A regional programme for training and research on tropical beekeeping and tropical bees in Costa Rica. *Bee World*, **77**, 3–7.
41) Sánchez-Castillo, C. P., Lara, J., Romero-Keith, J., Castorena, G., Villa, A. R., López, N., Pedraza, J., Medina, O., Rodríguez, C., Chávez-Peón, F. and James, W. P. T. (2001) Nutrition and cataract in low-income Mexicans: experience in an eye camp. *Arch. Latinoam. Nutr*., **51**, 113–121.
42) Vit, P. (2003) Cataratas oculares inducidas por radiaciones ultravioleta, VITAE Academia Biomedica Digital 2003, No. 15 [online] http://caibco.ucv.ve/caibco/CAIBCO/Vitae/Vitae Quince/Portada/home-vitae.htm (accessed 10.11. 2007)