14

Spectrophotometry as a Tool for Dosage Sugars in Nectar of Crops Pollinated by Honeybees

Vagner de Alencar Arnaut de Toledo[1,*], Maria Claudia Colla Ruvolo-Takasusuki[2], Arildo José Braz de Oliveira[3], Emerson Dechechi Chambó[1] and Sheila Mara Sanches Lopes[3]

[1]*Animal Science Department,*

[2]*Cell Biology and Genetics Department,*

[3]*Pharmacy Department, Universidade Estadual de Maringá, Maringá, Paraná*

Universidade Estadual de Maringá, Maringá,

Brazil

1. Introduction

The pollination by honeybees is important to the best performance of several crops. In this interaction plant-insect there is a change of reward between both organisms, and the sugar concentration in the nectar is a keyword. The spectrophotometry allows analyzing the type and the quantities of sugar in the nectar of flowers, and identifying varieties that are more attractive for pollinators.

The nectar is the reward for several pollinators, and the principal is the honeybee *Apis mellifera*. The nectar is produced from sap of phloem by active secretion that results in a solution of sugars like sucrose, fructose and glucose in varied proportion depending on the vegetal.

Besides sugars, other compounds of the nectar has importance for the coevolution between plants and their pollinators like amino acids, proteins, lipids and alkaloids and these may be toxic for visitors and, however, these compounds may have a role of protection against animals that withdraw nectar of flowers without an efficient pollination.

Several researches are carried out to evaluate the effect of crop pollination using honeybees and consequently the increase of productivity in agriculture. The visit and hoarding of nectar and pollen allows rise in grain production, or tasteful fruit with symmetric format.

The study of sugar from floral nectary is important for identification if a rise or decrease in quantity or nectar quality. The plant may secret a little bit of nectar, but with high sugar concentration, or unlike, secret more quantities, but with low sugar concentration. These differences in nectar may vary depend on pollinator visitation. However, the frequency of honeybees that visit flowers may contribute for rising nectar production like change the sugar proportion.

* Corresponding Author

Other factors that must be considered are secreted sugars: sucrose, glucose and fructose. The quantities of them may vary depending on variety and type of vegetal. Honeybees have preference for nectar with more sucrose concentration. Sugars present in nectar are related with honey quality that will be produced by honeybees and, finally will be commercialized.

The association of beekeeping and agriculture provide a rise in profits as for farmer as for beekeeper. However, the quantity and/or quality of the sugars in floral nectar like the pollination of cultivated crops by honeybees have an economic and social role well-established and significant currently.

The proposal of this chapter is perform a review about spectrophotometry in sugar dosages of nectar of the main cultivated crops and show the importance of this tool (spectrophotometry) to improve crop production by honeybee pollination contributing as for agriculture as for beekeeping.

1.1 Pollination and plant reproduction

The pollination process occurs in spermatophitic plants consists in pollen grain transfer to the stigma, which is the receptive part of feminine flowers of superior vegetal. The pollen grains are structures that contain the reproductive male cells, they are produced and storaged in anthers (part of male organ of flowers) until the deiscence, moment wherein are released. The indispensable factor in pollination process and that denotes the success is the need of ovule fertilization and subsequent fruit set and seeds formation.

The pollination of flowers beside the diaspore dispersion is a fundamental process in reproductive success of vegetal species. The involved animals in this process have an important role (Buckmann & Nabhan, 1997), then the efficience in the pollination process means rise in disponibility of food to the human being and animals.

The structure of plant (monoic or dioic) varies to the size and anatomic and physiological characterists of flower and their position in the plant, may occur the autopollination or cross pollination. This cross pollination provides a rise in gene flow between plants, spreading them and the results are favorable (Malerbo-Souza et al., 2004).

The animals that carried out the pollen transfer of anthers to the stigma flowers are known pollinators, and can be insects like bees, bettles, flies, butterflies, wasps and moth; birds - hummingbirds and parakeets; and small mammals - bats, rodents, and marsupials (Malagodi-Braga, 2005). Among pollinators, animals of Insecta class are the most important, and in the order Hymenoptera you can find the major number of them. Honeybees are the most important pollinators available in the nature.

Unlike of other insects that visit flowers only to collect its own food, honeybees visit a bigger quantity of flowers, besides of food to own survival, they harvesting pollen and nectar to feed their larvae and storaging (Müller et al., 2006). Futhermore, the higher efficiency of honeybees as pollinators is as much as by their number in the nature, as by better adaptation to floral complex structures like mouthparts and adapted body to imbibe the nectar of flowers and harvesting pollen, respectively (Proctor et al., 1996). The bees of Apidae family have higher distinction because their morphological traits are representative, like special structure to load pollen - corbicula, located in tibia of hind leg, similar to basket

– pollen is loaded in this structure in association with nectar or oil, absence of ventral scopa and long tongue (Teixeira & Zampieron, 2008).

Each group among animals that visit flowers is associated to some particular type of floral reward, that is the morphological traits of flowers reflect adaptation to diversified pollinators. The contemporaneous interactions between plants with flowers and insects can be because of long and closer coevolutionary relation (Backer & Hurd, 1968; Prince, 1997). This process of coevolution or the interaction between plants and pollinators is based on a system of mutual dependence. This system was detailed by the first time by Christian Konrad Spengel (1750-1816), in which plants show their rewards like nectar, pollen, oil and resins by floral arrangement, colour, size and odour of flowers, while the pollinators in change of provided resources by plants transfer pollen between flowers increasing the gene flow and promoting the diversification of the species, named by this as key mutualism (Morgado et al., 2002).

Plants, year by year, specialized in attract more efficient pollinators and make transportation of their reproductive cells, and therewith could be benefited with cross pollination. The disposition of flowers is an important factor that can be isolated in the branches or grouped in the same floral axis forming inflorescence, colour, odour, size, nectar, oil, pollen and resins.

The floral rewards provided by Angiosperms are required to attract pollinators, nectar seems the most searched in crop cultivated commercially, however, in searching by this reward many animals, mainly worker honeybees *Apis mellifera* have pollen adhered to their body, and so, later deposit accidentally their loads on the stigma of other flower of the same specie, performing indirectly the cross pollination.

Assays carried out in Marechal Cândido Rondon, Paraná, Brazil, with sunflower make clear worker honeybees that hoarding nectar are more frequent (mean 2.28 honeybees/capitulum) than pollen foragers (0.40 honeybees by capitulum) in anthesis period and schedule of higher visitation in the crop (Chambó et al., 2011). Other experimental results make clear more frequency of nectar foragers than pollen foragers in sunflower crop (Paiva et al., 2002; Teixeira & Zampieron, 2008).

Moreover, it must be considered that quantity of honeybee visitors to different species of superior vegetal can be related to concentration and volume of nectar in the flowers during all day (Pham-Delegue et al., 1990). Experiments with attractiveness sunflower genotypes show significative difference in relation to the number of honeybee visitation, mean of 3.40 (genotypes Helio 360 and Aguará) and 1.60 (genotype Multissol) visits of *A. mellifera* by capitulum in third day of anthesis (Chambó et al., 2011, in press). The researchers did not tested the concentration and volume secreted during all day, but assign to these causes the difference of sunflower genotypes assayed. In hybrid of ornamental coloured sunflower BRS-OASIS had an increase ($p < 0.05$) in four times in number of honeybee visitors using sucrose solution in two concentrations 5% and 7.5% as attractive. These solutions were pulverized on sunflower capitulum in relation to concentration of 2.5% (Martin et al., 2005).

Despite of efficiency of pollination process, it depends on numerous received visits by pollinators (Schirmer, 1985). Vidal et al. (2010) studying the pollination and set fruit in *Cucurbita pepo* by honyebees reported that percentage of set fruit was maximum (100%)

when the flowers received 12 visits of *A. mellifera*, corresponding to load of 1.253 pollen grains deposited on the stigma. In comparision, with two visits of honeybees, 174 pollen grains were deposited on the stigma, and the set fruit only of 50%. Other important factor is the attraction by pollen and nectar of male flower was arised by opening grade of nectary pore of flower (Vidal et al., 2010).

Models of pollen transfer depends on the specific pollinator rate about pollen remotion of anthers and deposition on the stigmas. The pollinators have a high remotion and low deposition (HRLD) of pollen on the stigma of flowers will benefite a plant wherever it is not better available pollinator. In case of pollinators have high remotion of pollen of anthers and high deposition (HRHD) of pollen on the stigmas also visit a plant population, the visits of pollinators HRLD can reduce the total pollen transfer. The HRLDs parasite the plants, displace the pollen grains that would delivery by HRHDs. When two visitors remove equal quantities of pollen, the pollinator more efficient will be that with higher delivery rate. In case of different quantities of remotion, the better do not depends on the deposition rates only, but another variables including schedule of visitation to pollen deposition (Thomson & Goodell, 2002).

The volume and sugar concentration of nectar, important factor in attraction of pollinators alos are known by varing between plant species and affect the answer of pollinator (Lanza et al., 1995). Besides, different varieties of the same species can range to the sugar concentration in the nectar (Free, 1993). In some vegetal species, for example, *Curcubita pepo,* the periodical remotion of nectar of the flower by pollinators do not arise the total volume of produced nectar by plant, then the nectar secretion is not stimulated or inhibited by successed harvesting of this reward. However, the nectar remotion of flowers can reduce or stimulate the process of secretion in several plant species.

The pollinator that drag out the spent time in flowers to nectar hoarding, specially if it is available, increase the probability of pollen deposition and, consequently, the pollination can be well successed. Nevertheless, cultivated commercially crops that do not stimulate the nectar production after several visits of pollinators can have an advantage in pollination process, so the number of honeybee visits to flowers is positively correlated to the nectar secretion in all flower duration (Vidal et al., 2006). The evaluation of nectar secretion rate is an important component in ecological studies related to the pollination process, mainly in that about flower-insect interaction.

2. Use of spectrophotometry in nectar analysis of plants

The quantitative analysis using spectrophotometric methods are widely used because have a good sensitivity, low analysis cost, easily handle, accessible equipments, justifying their application and efficiency for utilization in quantification analysis of several compounds.

The main spectrophotometric techniques used in sugar determination are based on reactions of these carbohydrates with colorimetric reagents, forming a colored complex that can be detected and quantified in a spectrophotometer.

The techniques used for total sugar quantification are anthrone method (Trevelyan & Harrison, 1952 in: Yemm & Wills, 1954), phenol-sulfuric (Dubois et al., 1956) and also can be used the 3-5-dinitrosalicylic acid - DNS modified (Miller, 1959). For reducing sugar

determination, also can be used the technique of DNS and the reaction with p-hydroxybenzoic acid hydrazide - *PAHBAH* (Blakeney & Mutton, 1980).

For specific determination of some sugars also are available some other methods. Fructose can be determinated by cysteine/tryptophan/sulphuric acid method (Messineo & Musarra, 1972) and resorcinol method (Roe et al., 1949). Sucrose can be determined by anthrone method after destruction of monosaccharides with KOH (Sala Jr. et al., 2007). Besides these methods, the glucose, fructose and sucrose can be selectively determined by methods using enzymatic reactions (Moernan et al., 2004; Amaral et al., 2007).

3. Total sugar determination

3.1 Phenol-sulphuric method (Dubois et al., 1956)

This method is based on the fact that simple or complex sugar and their derivatives, including methyl esters with free reducing groups or potentially free, when treated with phenol and concentrated sulphuric acid will generate a yellow-orange colour, the reaction is sensible and this colour is stable. The method is simple, quick, sensible, and the results are reproductible.

The changing in colour of solution is measured in visible range and proportional to the quantity of sugar inside the sample.

The carbohydrates are hydrolyzed under heating in strongly acid pH, this reaction produces furan derivative compounds, that when condensed with phenolic compounds produce coloured substances (Figure 1).

The total sugar concentration is determined by spectrophotometry in 490 nm wavelength. Sensitivity of this method range from 10 to 100μg of total sugar and the quantification is made from calibration curve using glucose or pentose as standard and calculation are performed by equation of the linear regression obtained from calibration curve.

3.1.1 Methodology

Reagents: phenol solution 5% (w/v) and concentrate sulphuric acid (95%, p/v).

Get an aliquot of 100μg.mL^{-1} from the sample, add 0.5mL of phenol solution 5%, shake in vortex, add 2.5mL, shake again and keep in water-bath 25°C for 15 minutes. After this period, read the absorbance in spectrophotometer at 490nm.

3.2 Anthrone method (Dreywood, 1946)

The anthrone is the reduction product of anthraquinone and was recognized first as specific reagent for several carbohydrates by Dreywood (1946) because sugar solution in concentrate sulphuric acid form the blue-greenish colour characteristic and since anthrone has been used widely as suitable and specific reagent for sugar colorimetric dosage.

The anthrone reaction is based on hydrolitic and dehydrating action of concentrate sulphuric acid on carbohydrates, in which glicosidic linkages are broken releasing free reducing sugar that are dehydrated and converted to furfural by pentoses and hydroxymethylfurfural by hexoses (Figure 1).

These substances are condensed with anthrone to hydroxyanthracene (9,10-dihydro-9-oxo anthracene) forming a blue-greenish product that have absorption maximum at 620nm.

The sensitivity of this method is from 0 to 100µg.mL^{-1}. Glucose solution is used as standard to build calibration curve and get the straight line equation to quantify samples.

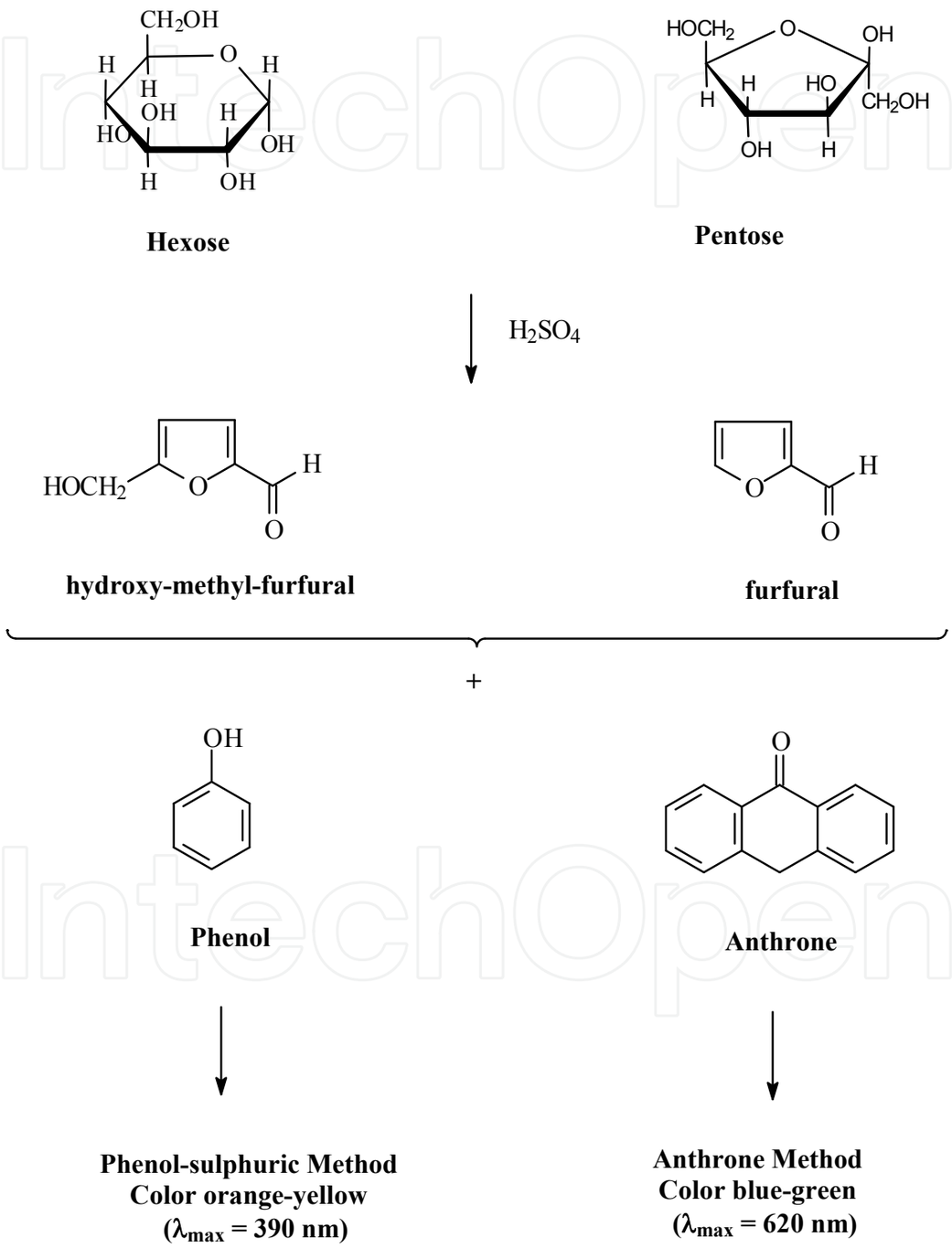


Fig. 1. Reaction of formation of the coloured complex in phenol-sulphuric and anthrone methods.

3.2.1 Methodology

Reagents: anthrone solution 0.2% (0.2g anthrone em q.s. to 100mL of sulphuric acid 95%). This solution must be kept at rest for 30 minutes with sporadic agitation until the solution to be clear, the reagents must be used in 12 hours.

Get an aliquot of 100µg.mL^{-1} from the sample, add 2mL of the anthrone, leave at ice bath and hereupon cool in cooled water and read the absorbance in spectrophotometer at 620nm.

4. Reducing sugar determination

4.1 3,5-dinitrosalicylic acid method (Miller, 1959)

This method was first mentioned by Summer & Sisler (1944) and modified by Miller (1959), with this technique there is a possibility to dosage reducing sugar as total sugar.

The sugar act as a chemical reductor due free aldehyde group or ketone group presence in its molecule. In an alkaline medium, the reducing sugars are able to reduce the 3-5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, wherever, the aldehyde group is oxidized to aldonic acid (Figure 2). The 3-amino-5-nitrosalicylic acid is a orange color product, and the intensity of the color depends on the concentration of the reducing sugar. The sodium hydroxide provides the glucose reaction with 3-5-dinitrosalicylic acid by medium alkalinization.

Besides to 3-5-dinitrosalicylic acid, it is also used in this method the Rochelles salt (Potassium sodium tartrate), phenol, sodium bisulfite, and sodium hydroxide. The phenol optimize the quantity of the colour produced and sodium bisulfite stabilize the colour in the phenol presence (Miller, 1959).

The sensitivity of the method is from 100 to 500µg.mL^{-1} of reducing sugar. A standard glucose or fructose solution is used to build the calibration curve and get the straight line equation to quantify samples.

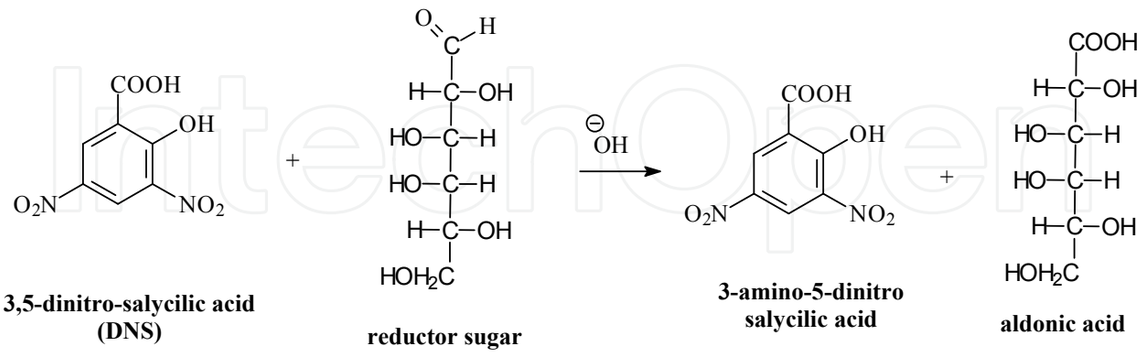


Fig. 2. Reaction of reducing sugar with 3,5-dinitro-salycilic acid reagent.

4.1.1 Methodology

Reagents:

Reagent A – Weight 2.5g DNS (3,5 dinitrosalicylic acid) and add 50mL NaOH 2M.

Reagent B – Weight 75g Potassium sodium tartrate (Rochelle salt), add 125mL of destiled water. Shake under heating until total dissolution.

Subsequently, add reagent A over the B, homogenize under heating until dissolve completely, and after cool the mix, complete the volume of the solution to 250mL.

Get an aliquot 500µg.mL^{-1} of the sample. Add 1mL of DNS, shake in vortex and let in a boiling bath during 5 minutes, put the material in ice bath until cool, add 3.75mL of destiled water, shake again, and read the absorbance in spectrophotometer at 540nm.

An adaptation of the DNS method to determine the total sugar can be getting from a previous hydrolysis before dosage. The hydrolysis is made with 0.5mL HCl concentrate and incubation in water bath at 60°C for 10 minutes. After then, the solution must be neutralized with NaOH 2M and cooled with ice bath until room temperature.

The procedure for total sugar quantification follow the same described for reducing sugar used above.

4.2 Hydrazide method of p-hydroxy-benzoic acid (Blakeney & Mutton, 1980)

This method is based on determination of reducing sugar before and after the digestion by invertase using acid for p-hydroxy benzoic acid hydrazide – PAHBAH. In this method, with some modifications, there is possible to perform the colorimetric determination of glucose, fructose and sucrose, during this analysis procedure with invertase. The major subject of the methodology described in this chapter is for determination of reducing sugar. The p-hydroxy benzoic acid hydrazide also has the advantage that glucose and fructose when reacting produce the same intensity of colour, and then all free monossacharides present in the sample can be determined.

4.2.1 Methodology

Reagents:

Reagent A: 10g p-hydroxy benzoic acid dissolved in 60mL water, add 10mL hydrochloride acid and complete volume to 200mL.

Reagent B: 24.9g trisodium citrate in 500mL water, 2.2g calcium chloride, 40g sodium hydroxide, and to complete the volume to 2.000mL.

In the day of analysis, mix reagents A and B in proportion of 1:10, after keep at 4°C.

Get an aliquot 50µg.mL^{-1} of the reducing sugar sample, add 5mL p-hydroxy benzoic acid hydrazide reagent (mixed in the same day) shake in vortex, incubate in water bath at 100°C by six minutes, cool until room temperature, and read the absorbance in spectrophotometer at 410nm.

5. Fructose determination

5.1 Sulphuric acid-Cysteine-Tryptophan method (Messineo & Edward Musarra,1972)

Fructose is dehydrated in acid medium for formation of furfural derivative, which complexs with cysteine hydrochloride to produce a chromophore at starting of green color that is

unstable. Immediately, this first chromophore formed after react with tryptophan hydrochloride forming now a chromophore of pink colour that has greater stability chemical than the first and stability of 48 hours.

The reaction is also sensitive because can detect a low quantities like 1µg of fructose. The reaction also is specific because aldohexoses, aldopentoses, and ketopentoses do not interfere, however these compounds do not react even if its concentrations are higher, as 5mg.mL^{-1}. The sensitivity of the method is from 1 to 50µg of fructose. A standard solution of fructose is used to build the calibration curve and to get the equation of the linear regression to quantity samples.

5.1.1 Methodology

Reagents: sulphuric acid 75%, cysteine hydrochloride 2.5%, tryptophan solution in hydrochloride acid to formation of tryptophan hydrochloride (100µg.mL^{-1} in HCl 0.1M).

Getting an aliquot 50µg.mL^{-1} of the sample, add 2.8mL of sulphuric acid 75%, shake in vortex, add 0.1mL cysteine hydrochloride solution 2.5%, shake in vortex again, let in water-bath 45-50°C for 10 minutes, cool at room temperature and add 1mL tryptophan hydrochloride solution, shake again in vortex.

This sequence must be followed rigorously during assay because the formation of the final chromophore depends on the initial formation of the first formed chromophore by complexation with cysteine hydrochloride. After that, read the absorbance in spectrophotometer at 518nm.

5.2 Resorcinol method (Roe et al., 1949)

This reaction follows the same theoretical principles in which there is formation of furfural from hexoses and hydroxy-methyl-furfural (HMF) and from aldopentoses by acid dehydration (Fig. 1). These two products, singly are colorless, however, it is necessary a phenolic compound addition in the medium to develop a colored compound, in this case redness. This technique is firstly mentioned by Roe (1934), with some posterior modifications by Roe et al. (1949), becoming a quick reaction and with stable color. The reaction uses the hydrochloride acid (HCl) for carbohydrates dehydration and the resorcinol is the phenolic compound that reacts with furfural and HMF.

This test allows distinguish aldoses from ketoses because the reaction with ketoses is faster and more intense than aldoses. Therefore, the formation of the furfural is easier than HMF formation.

The sensitivity of the method ranges from 10 to 80µg.mL^{-1} fructose. A standard fructose solution is used to build the calibration curve and to get the equation of the linear regression to quantity samples.

5.2.1 Methodology

Reagents: Resorcinol reagent, 1g resorcinol and 0.25g thiourea in 100mL. This solution must be kept in the dark to keep its stability. Hydrochloride acid is diluted as 5mL HCl and 1mL water.

Getting an aliquot 80μg as maximum of the sample, add 0.5mL resorcinol reagent, shake in vortex, add 3.5mL hydrochloride acid, shake again, let in water bath at 80°C for 10 minutes, after that, read the absorbance in spectrophotometer at 520nm.

6. Enzymatic methods for sugar determination

There are several enzymatic methods for determination of the three principal sugars individually present in nectar - fructose, glucose, and sucrose, and in biological samples like plasma, blood, and urine. A lot of these methods are commercialized in kits and can be used successfully for rapid determination of the sugar from natural products samples. These kits are precise and sensitive, which enable rapid analysis and reliable results.

6.1 Glucose oxidase (Amaral et al., 2007)

The enzyme glucose oxidase is used for quantitative and enzymatic determination of the glucose in food and other materials. The enzyme glucose oxidase test is widely used because it is cheap, stable, and by its specificity well established for glucose.

In this reaction, the glucose is oxidized to gluconic acid and hydrogen peroxide by enzyme glucose oxidase. The hydrogen peroxide reacts with ortho-dianisidine in the presence of peroxidase enzyme to form a colored product (Figure 3). The compound of the ortho-dianisidine oxidation reacts with sulphuric acid to form a coloured product more stable. The intensity of pink colour measured in 540nm is proportional to the glucose concentration in the sample.

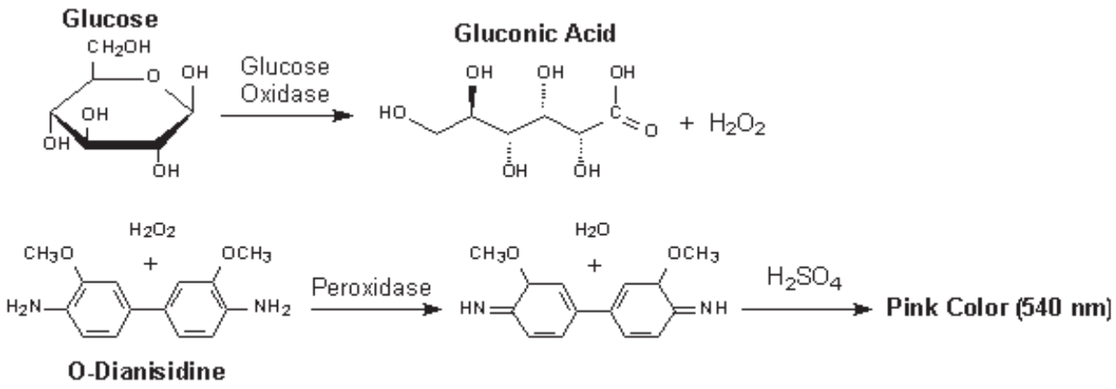


Fig. 3. Reactions of the enzymatic method of glucose oxidase.

6.2 Hexokinase method for simulteneous determination of glucose and fructose (Moerman et al., 2004)

This method is adequate for determination of monosaccharides glucose and fructose.

The principal enzyme of the method is hexokinase. This enzyme catalyzes the fosforilation of the glucose in glucose-6-phosphate, here upon the second enzyme the glucose-6-phosphate dehydrogenase together with cofactor nicotinamide adenine dinucleotide (NAD) oxidized glucose-6-phosphate to gluconate-6-phosphate and the NAD is reduced to NADH,

according to stoichiometry of the second reaction (Figure 4), the spectrophotometric quantity of NADH is corresponding to glucose quantity.

Analysis of glucose is according to the following principle: hexokinase, a first enzyme, catalyzes the phosphorylation of glucose to glucose-6-phosphate, with the participation of the enzyme glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide (NAD) is further specifically oxidized to gluconate-6-phosphate. According to the stoichiometry of the last reaction (Figure 4), the photospectrometrically quantified amount of reduced nicotinamide adenine dinucleotide (NADH) is representative for the amount of glucose. Fructose is always determined subsequently to the glucose determination. Fructose undergoes phosphorylation to fructose-6-phosphate, with the same enzyme hexokinase, which is further converted to glucose-6-phosphate with phosphoglucose isomerase. Further oxidation to gluconate-6-phosphate as described above generates a supplementary amount of NADH that is stoichiometric with the amount of fructose.

All methodology of this analysis is performed following instructions of each enzymatic kit. For these enzymatic analysis must carried out the assays criteriously, because the order of addiction of reagents, the time of analysis and reading on spectrophotometer are determinants for an adequate analysis.

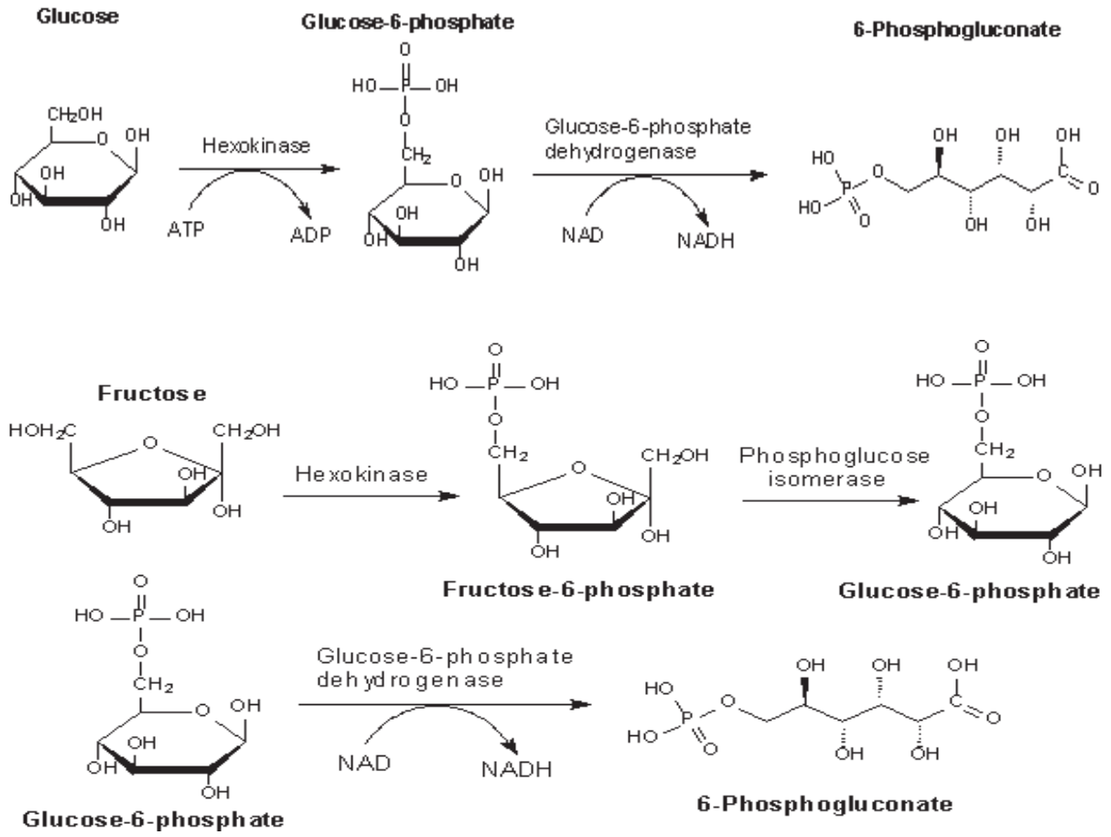


Fig. 4. Enzymatic reactions in enzymatic methods using hexokinase.

7. Sugar concentration in nectar

Nectar is considered the main reward to pollinator (Delaplane & Mayer, 2000) and its sugar concentration is associated to different pollinators, wherever the frequency and duration of visits depend on rate of nectar production (Biernaskie et al., 2002; Shafir et al., 2003; Nicolson & Nepi, 2005).

Toledo et al. (2005) reported total sugar concentration presents variation during the day in *Macroptilium atropurpureum* Urb., and can be related to number of visitor insects, specially, bees that collect nectar and pollen. In *Citrus sinensis*, it was verified that the high sugar concentration is an attractive, in special for *Apis mellifera*, and the availability of concentrated nectar during the day keeps the attractivity to pollinators (Malerbo-Souza et al., 2003). Therefore, the high quantity of nectar can leads to greater pollination rate by increasing in number of visitors bees (Silva & Dean, 2000).

Sugars are principal components of the floral nectar (Baker & Baker, 1973; Baker, 1977). The three most common sugar in nectar are sucrose, glucose and fructose in varying proportions (Freeman et al., 1985; Endress, 1994; Proctor et al., 1996; Baker et al., 1998). The amount of sugar secreted by flowers and consumed by pollinators cause a variation in sugar concentration of the flowers, during their anthesis period. Floral nectar consists of sugar pure solutions, specially glucose, sucrose and fructose (Roberts, 1979), however it can be found traces of oligosacchrides (Harbone, 1998).

Wykes (1952a) reported two oligosaccharides in nectar composition, the trisaccharide raffinose, and disaccharide melibiose in some nectaries. In some varieties of clover the presence of disaccharide maltose also was identified, however, this maltose can be a contaminant from aphids (Furgala et al., 1958). Taufel & Reiss (1952) confirmed that sucrose, glucose, and fructose, sugars promptly accepted by honeybees are current compounds present in nectar but another sugars can be present.

Besides, fructose and glucose, the presence of the monosaccharide D-galactose, in very low quantities (traces) also already was related in honey samples (Goldschmidt & Burket, 1955). However, it is important to emphasize that this monosaccharide when in its free form is considered a toxic compound to the honeybees (Siddiqui, 1970). Moreira & De Maria (2001) reviewed about carbohydrates in honey, and reported several di-, tri-, and oligosaccharides presented in honey and they came from nectar.

High-fructose is commonly used as sugar substitutes in processed foods, especially in soft drinks, mainly for economical reasons (Long, 1991). These products high-fructose corn syrups (HFCS) are obtained by enzymatic isomerization of corn syrups by both acid and enzymatic hydrolysis of cornstarch. Three enzymes are needed to transform cornstarch into the simple sugars glucose and fructose, α-amylase, glucoamylase, and glucose-isomerase. Fructosyl-fructoses were mainly detected in honey from honeybees fed with high-fructose corn syrups but not from those honeys coming from free-flying foragers or workers fed with sugar syrup (Ruiz-Matute et al., 2010).

Percival (1961) examined 889 plant species and found three pattern of carbohydrates to the nectar: a) nectar with high sucrose, nectar with similar quantities of sucrose, glucose and fructose; and nectar with high glucose and fructose. The nectar with sucrose dominant was associated to flowers of long tubes in which the nectar was protected (clovers), wherever the

opened flowers had generally only glucose and fructose. These reports confirmed early researches (Wykes, 1953; Bailey et al., 1954) that suggested a relation between three monosaccharides and different species with flower. In another research, the nectar was divided in four different classes in function to sucrose/hexose rate - S/H: sucrose dominant - S/H >0.999, rich in sucrose - 0.5<0.999, rich in hexose - 0.1<0.499 and hexose dominant - S/H<0.1 (Baker & Baker, 1983).

In the research of Alves (2004) and Alves et al. (2010), the means of sucrose.hexose^{-1} (S/H) per flower for all treatments were: 0.91µg.µL^{-1}, for covered area with Africanized honeybee colony – rich in sucrose; 0.74µg.µL^{-1}, semicovered area with free insects visitation – rich in sucrose; 0.86µg.µL^{-1}, uncovered area with free insect visitation – rich in sucrose; and 3.05µg .µL^{-1}, for covered area without Africanized honeybee colony – sucrose dominant. However, Severson e Erickson (1984) reported in several cultivars of soybean values from 1.2:1.0:1.4 to 1.2:1.0:6.7, with sucrose predominance, which sucrose concentration in nectar ranged from 97 to 986µg.µL^{-1}, these means are higher than those reported by Alves et al. (2010) who found 12.06µg.µL^{-1}. This range suggests that sugar concentration in soybean nectar is influenced by other environment factors independently of pollinator action. Robacker et al. (1983) reported that edaphic and climatic factors affect the number of flowers and another floral characteristics during soybean growing. So, the environmental conditions that generate an increase in number and size of flowers, higher anthesis period, colourness more intense, and also greater nectar production are the factors responsible by became flowers more attractive to honeybees (Alves et al., 2010).

Cruden et al. (1983) suggested that the maximum nectar accumulation occurs before or at the beginning of pollination activity. Such fact can be verified in siratro, since the highest sugar concentration was found at 8:30 a.m. (Figure 5) time in which the bee visitation started (Toledo et al., 2005). Variations in the siratro nectar sugar content measured along the day were observed (Figure 6 – Toledo et al., 2005) and probably associated with the intensity of foraging by honeybees, which is directly related to the nectar quantity and quality (Heinrich, 1979; Hagler, 1990) or to its sugar composition (Waller, 1972; Abrol & Kapil, 1991).

In flowers exposed to pollinators, it is possible that nectar secretion ceases if there is not pollinator in the area or can be reabsorbed in old or pollinated flowers (Cruden et al., 1983). A nectar production without reabsorption may be have an impact on reproductive biology (Galleto & Bernardello, 1995). Therefore, plants reabsorb nectar from aging flowers and utilize its carbon in developing seeds and this is a reproductive advantage (Zimmerman, 1988).

Chiari et al. (2005) studying the pollination of Africanized honeybees on soybean flowers (*Glycine max* L. Merrill) var. BRS 133, measured through the manual refractometer the sugar concentration as total solids and concluded that data found presented a big uniformity, different of the results obtained by Sheppard et al. (1978) that observed big variations in these concentrations and attributed these differences to the variation in the soil composition and other environmental conditions, like precipitation. Despite this, the mean values found by Chiari et al. (2005) were 21.33 ± 0.22% in uncovered area and 22.33 ± 0.38% in covered area with honeybees and differed to each other (P=0.0001). Besides, the medium amounts of total sugar and glucose measured in the nectar of the flowers were 14.33 ± 0.96mg/flower and 3.61 ± 0.36mg/flower, respectively, in the same research.

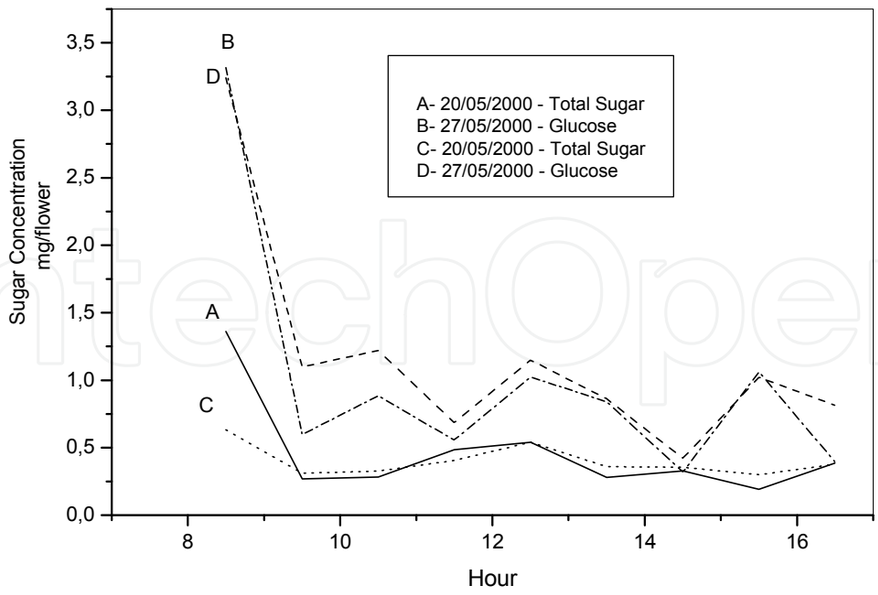


Fig. 5. Total sugar and glucose contents in nectar of siratro flowers along two-day period - reprinted from Toledo et al. (2005) with permission.

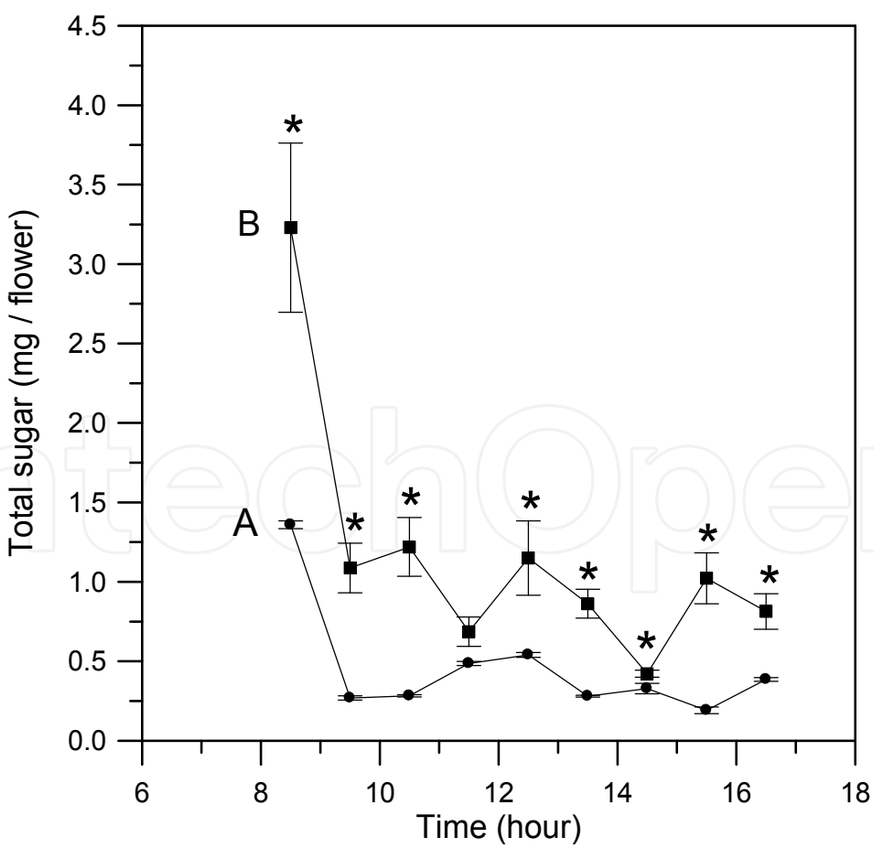


Fig. 6. Total sugar contents in nectar of siratro flowers along two-day period - reprinted from Toledo et al. (2005) with permission.

The study of 25 canola (*Brassica napus x Brassica campestris*) varieties carried out by Kevan et al. (1991) demonstrated that 23 of them had 0.95 or more glucose:fructose rates in their nectar. The same authors reported too that only three varieties had glucose in smaller quantities and none of the samples had detectable quantities of sucrose. Davis et al. (1998) reported higher glucose/fructose rate in lateral chambers than median in Brassicaceae.

Chromatography in paper showed that the nectar from siratro (*Macroptilium atropurpureum* Urb.) is constituted exclusively of glucose, as may be seen in Figure 5 (Toledo et al., 2005). It can be explain the low percentage of honeybee visit on siratro flower - 4%, wherever the another bees were *Trigona spinipes* - 24%, *Bombus morio* - 8%, *Euglossa sp* – 20%, *Megachilidae* - 12%, *Pseudaugochloropsis graminea* – 8% and *Halictidae* - 8% (Vieira et al., 2002). From this, it can be concluded that the main visitor and pollinator in siratro was *Euglossini*, however, *Trigona spinipes* perfurated the external part of the flower avoiding the contact with the pollen grains (Vieira et al., 2002).

Alves (2004) reported a variation of total sugar concentration in soybean nectar var. Codetec 207 from 21.33 to 27.47%, and the higher total sugar concentration was observed in covered area with Africanized honeybee colony. The sucrose concentration ranged from 9.63 to 13.61%, and the higher sucrose concentration was observed in covered area with Africanized honeybee colony. The glucose concentration in this variety was very low. The fructose concentration ranged from 7.93 to 13.75%, so the covered area with Africanized honeybee colony presented higher concentration too. Alves (2004) suggested that Africanized honeybees estimulated the sugar secretion in soybean nectar var. Codetec 207 (Table 1).

Aproximately, 20% of all food-crop production and about 15% of seed crops require the help of pollinators for full pollination (Klein et al., 2007), but Kevan & Phillips (2001) reported that aproximately 73% of cultivated vegetals in the world would be pollinated by some bee species. Gallai et al. (2009) reported a bioeconomic approach, which integrated the production dependence ratio on pollinators, for the 100 crops used directly for human food worldwide as listed by FAO. The total economic value of pollination worldwide amounted to €153 billion, which represented 9.5% of the value of the world agricultural production used for human food in 2005. The honeybee is the most common insect used as agricultural pollinator in many parts of the world like Europe and United States.

In United States, farmers rent more than 2 million honeybee colonies every year for pollination, but the honeybee is being threatened by several problems like Colony Colapse Desorder (James & Pitts-Singer, 2008). The cost of renting bees is up from US$50 per hive in 2003 to US$ 140 per hive in 2006 (Sumner & Boriss, 2006). Some crops require five to seven hives per hectare. In addition, no all crops are well pollinated by honeybees. For example, tomatoes require buzz pollination, which honeybees cannot achieve and alfalfa flowers are not properly worked by honeybees. Fortunately, honeybees are not the only bees that make good pollinators (James & Pitts-Singer, 2008).

Therefore, Greenleaf & Kremen (2006) reported that behavioral interactions between wild and honeybees increase the pollination efficiency of honeybees on hybrid sunflower up to 5-fold, effectively doubling honeybee pollination services on the average field. These indirect contributions caused interspecific interactions between wild and honeybees were more than five times more important than the contributions wild bees make to sunflower pollination directly.

		H=19.88		DF=3		KW=0.0002
Total Sugar	x±se (%)	n*	Rx	T2	T3	T4
covered area with honeybee colony	27.47±1.37	52	137.16	ns	0.0009	0.0008
semi-covered area	26.80±1.81	32	137.81		0.0039	0.0033
uncovered area	21.33±0.86	74	98.24			ns
covered area without honeybee	21.74±1.05	66	96.79			
		H=13.87		DF=3		KW=0.0031
Sucrose	x±se (%)	n	Rx	T2	T3	T4
covered area with honeybee colony	13.01±1.26	51	135.62	ns	0.0004	ns
semi-covered area	12.74±1.49	33	123.61		0.0028	ns
uncovered area	09.63±1.18	78	93.60			0.0366
covered area without honeybee	12.86±1.49	65	116.65			
		H=0.78		DF=3		KW=0.8531
Glucose	x±se (%)	n	Rx	T2	T3	T4
covered area with honeybee colony	0.71±0.05	81	135.62	ns	ns	ns
semi-covered area	0.78±0.09	52	123.61	ns	ns	ns
uncovered area	0.98±0.11	97	93.60	ns	ns	ns
covered area without honeybee	0.95±0.10	83	116.65	ns	ns	ns
		H =12.26		DF=3		KW=0.0065
Fructose	x±se (%)	n	Rx	T2	T3	T4
covered area with honeybee colony	13.75±1.36	51	59.30	ns	ns	0.0016
semi-covered area	13.29±1.38	33	58.42		0.0701	0.0075
uncovered area	10.72±1.05	78	46.33			0.0390
covered area without honeybee	7.93±1.32	65	34.92			

*n- sample size; x-Averages; se-standard error; H - H test; DF-degrees of freedom; KW-Kruskal-Wallis Probability; Rx-Medium position; ns-non significant and probability of the interactions (T1, T2, T3 and T4 vs T2, T3 and T4)

Table 1. Means of total sugar, sucrose, glucose and fructose concentration (%), in soybean nectar (Glycine max L. Merrill) var. Codetec 207 for covered area with honeybee colony (T1); Semi-covered area (T2); uncovered area (T3) and covered area without honeybee colony (T4) – Reprinted from Alves (2004) and Alves et al. (2010) with permission

Analysis of sugar composition in nectar can be used for detecting variation between flowers or nectaries from different taxonomic varieties, and consequently generate differences in type and frequency of visitation of pollinators. Alves et al. (2010) studied the total sugar concentration in soybean nectar (*Glycine max* L. Merrill) var. Codetec 207 by spectrophotometry, using the general method for carbohydrates determination by phenol-sulphuric technique (Dubois et al., 1956).

The research of Alves et al. (2010) was carried out in soybean plants in cages of 24m² covered with an Africanized honeybee colony inside in which, semicovered area with free insect visits, uncovered area, covered area without Africanized honeybee colony. Each treatment was five repetitions. In this research, it was emphasized greater total sugar

concentration in covered area with Africanized honeybee colony, reduction of sucrose concentration in uncovered area, and lower fructose concentration in covered area without honeybee colony and uncovered area. Besides, none difference among treatments in relation to glucose concentration. So, the high total sugar concentration observed in nectar of soybean var. Codetec 207, in covered area with Africanized honeybee colony suggests that the presence of *Apis mellifera* influenced in this composition, even though a variety with high grade of autopollination. However, the low fructose concentration in uncovered area can be related with low density of honeybees recorded or low presence of another preferential pollinator of fructose. For some researchers, the pollinators can affect the nectar composition (Canto et al., 2008; Herrera et al., 2009). In *Helleborus foetidus*, for example, some species of *Bombus* unchain modifications in sugar composition in nectar reducing the sucrose percentage, and rising the fructose and glucose percentage (Canto et al., 2008).

8. Conclusion

Nectar is an important floral reward for the bee visitation in the flowers. That food is converted in honey into the hive and used as an energy source for the workers. The bee-plant interaction is essential for the maintenance of genetic variability of plants as well as increased production of grains and fruits on a commercial scale.

Honeybees are very important for the pollination of both cultivated and native plants, and then understand the relationship between the collection of floral products (nectar, pollen, resins and oils), biology and behaviour of these insects help making better use for making bee products and agricultural products. Spectrophotometry is a tool to quantify and identify the components of floral products used by bees, especially nectar. These tests allow checking the correlation between the type and intensity of nectar in flower visitation by bees.

The methods employed in this molecular tool began to be developed in the early 50s of last century. Currently, the main analyses performed with the nectar spectrophotometry are: total sugars, reducing sugars, fructose determination (furfural and resorcinol), glucose oxidase (glucose determination in foods), hexokinase (glucose and fructose) and sugar concentration in nectar. As well as, in honey are made several analyses too, like diastase index, hydroxymethylfurfural and others.

Among the tests that can be done is determining sugar concentration in the nectar. The quantification of these sugars allows developing a series of studies associated with the floral visitation by bees collecting nectar and pollination of several species of cultivated plants. These studies contribute to the use of *A. mellifera* to assist beekeepers in increasing the honey production and the farmer in agricultural production. The association between agriculture and beekeeping has been demonstrated by studies presented that showed a positive association between the type of nectar produced by plant and intensity collection by honeybees. Much is still to be undertaken because the number of plant species studied that are pollinated by bees is limited in relation to the number of known species.

Especially in tropical regions, stingless native bees must be preserved and have great potential apicola and sustainable that is not operated, besides to the physicochemical analysis of the sugar composition of honey produced by these bees is not well known and do not have legislation around the world. Floral preferences of native stingless bees are not

established yet. Spectrophotometry is an important tool to quantify and identify these components and contribute to knowledge about the interaction of stingless bees and flowers species in neotropical regions, besides to continue to be used in bee products chemical determination.

Finally it is worth mentioning that the spectrophotometry should be more standardized and published as an analysis tool to better use of the bees by the beekeepers in beekeeping industry and in agricultural production.

9. Acknowledgements

To the National Council for Scientific and Technological Development (CNPq), process number 479329/2009-5, and Coordination of Improvement Staff (CAPES) for their financial support.

10. References

Abrol, D.P. & Kapil, R.P. (1991). Foraging strategies of honeybees and solitary bees as determined by nectar sugar components. *Proceedings of the Indian National Academy of Sciences,* Vol. 57-B, p. 127-132, ISSN 0019-5588

Alves, E.M. (2004). *Polinização e composição de açúcares do néctar de soja (Glycine max L. Merrill) variedade Codetec 207.* Master dissertation, Universidade Estadual de Maringá, Maringá, Brasil. 57p.

Alves, E.M.; Toledo, V.A.A.; Oliveira, A.J.B.; Sereia, M.J.; Neves, C.A. & Ruvolo-Takasusuki, M.C.C. (2010). Influência de abelhas africanizadas na concentração de açúcares no nectar de soja (*Glycine max* L. Merrill) var. Codetec 207. *Acta Scientiarum – Animal Science,* Vol. 32, No. 2, pp. 189-195, ISSN 1807-8672

Amaral, L.I.V.; Gaspar, M.; Costa, P.M.F.; Aidar, M.P.M. & Buckeridge, M.S. (2007). Novo método enzimático rápido e sensível de extração e dosagem de amido em materiais vegetais. *Hoehnea*, Vol.34, p. 425-431, ISSN 0073-2877

Bailey, M.E.; Fieger, E.A. & Oertel, E. (1954). Paper chromatographic analyses of some southern nectars. *Gleanings in Bee Culture,* Vol. 82, p. 7-8, ISSN 0017-114X

Baker, H.G. & Hurd, Jr., P.D. (1968). Intrafloral ecology. *Annual Review of Entomology*, Vol.13, No.1, p. 385-414, ISSN 0066-4170

Baker, H.G. (1977). Non-sugar chemical constituents of nectar. *Apidologie*, Vol. 8, p. 349-356, ISSN 12979678

Baker, H.G. & Baker, I. (1973). Some anthecological aspects of the evolution of nectar producing flowers, particularly amino acid production in nectar. In: Heywood, V.H. (Ed.) *Taxonomy and ecology.* Academic Press, ISBN 0-12-346960-0, London, UK

Baker, H.G. & Baker, I. (1983). A brief historical review of the chemistry of floral nectar. In: Bentley, B. & Elias, T.S. (Eds.) *The biology of nectaries*. Columbia University Press, ISBN 978-0-231-04446-2, New York, USA

Baker, H.G.; Baker, I. & Hodges, S.A. (1998). Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. *Biotropica*, Vol. 30, p. 559-586, ISSN 0006-3606

Biernaskie, J.M.; Cartar, R.V.; Hurly, T.A. (2002). Risk-averse inflorescence departure in hummingbirds and bumble bees: could plants benefit from variable nectar. *Oikos,* Vol. 98, No. 1, p. 98-104, ISSN 1600-0706

Blakeney, A.B. & Mutton, L.L. (1980). A simple colorimetric method for the determination of sugars in fruit and vegetables. *Journal of the Science of Food and Agriculture*, Vol.31, No.9, p. 889-897, ISSN 1097-0010

Buckmann, S.L. & Nabhan, G.P. (1997). *The forgotten pollinators*, Island Press, ISBN 1559633522, Washington, DC, USA

Canto, A.; Herrera, C.M.; Medrano, M.; Perez, R. & Garcia, I.M. (2008). Pollinator foraging modifies nectar sugar composition in *Helleborus foetidus* (Ranunculaceae): an experimental test. *American Journal of Botany*, Vol. 95, No. 3, pp. 315-320, ISSN 1537-2197

Chambó, E.D.; Garcia, R.C.; Oliveira, N.T.E. & Duarte, Jr., J.B. (2011). Honey bee visitation to sunflower: effects on pollination and plant genotype. *Scientia Agricola*, Vol.68, No.6, ISSN 0103-9016

Chiari, W.C.; Toledo, V.A.A.; Ruvolo-Takasusuki, M.C.C.; Attencia, V.M.; Costa, F.M.; Kotaka, C.S.; Sakaguti, E.S. & Magalhaes, H.R. (2005). Floral biology and behavior of Africanized honeybees *Apis mellifera* in soybean (*Glycine max* L. Merrill). *Brazilian Archives of Biology and Technology,* Vol. 48, No. 3, p.367-378, ISSN 1516-8913

Cruden, R.W.; Hermann, S.M. & Peterson, S. (1983). Patterns of nectar production and plant-pollinator coevolution. In: Bentley, B. & Elias, T. (Eds.). *The biology of nectaries.* Columbia, ISBN 023104461, New York, USA, p. 80-125

Delaplane, K.S.; Mayer, D.F. (2000). *Crop pollination by bees.* CABI Publishing, ISBN 9780851994482 London, UK

Dreywood, R. (1946). Qualitative test for carbohydrate materials. *Industrial and Engineering Chemistry Analytical Edition*, Vol.18, p. 499-504, ISSN 0096-4484

Dubois, M.; Giles, K.A. & Hamilton, J.K. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, Vol. 28, p. 350-356, ISSN 0003-2700

Endress, P.K. (1994). *Diversity and evolutionary biology of tropical flowers.* Cambridge, ISBN 0-521-56510-3, Cambridge, USA

Free J.B. (1993). *Insect pollination of crops*. Academic Press, ISBN 0122666518, London, UK

Freeman, C.E.; Worthington, R.D. & Corral, R.D. (1985). Some floral nectar-sugar compositions from Durango and Sinaloa, Mexico. *Biotropica*, Vol. 17, p. 309-313, ISSN 0006-3606

Furgala, *B.*; *Gochnauer*, *T.A.* & *Holdaway*, *F.G. (1958)*. Constituent sugars of some northern legume nectars. *Bee World, Vol. 39, p.* 203-205, ISSN 0005-772X

Galetto, L. & Bernardello, G. (1995). Characteristics of nectar secretion by *Lycium cestroides*, *L. Ciliatum* (Solanaceae), and their hybrid. *Plant Species Biology*, Vol. 11, p. 157-163, ISSN 1442-1984

Gallai, N.; Salles, J.-M; Settele, J. & Vaissière, B.E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics,* Vol. 68, p. 810-821, ISSN 0921-8009

Goldschmidt, S. & Burkert, H. (1955). Observation of some heretofore unknown sugars in honey. *Zeitschrift für Physiologische Chemie*, Vol. 300, p. 188, ISSN 0018-4888

Greenleaf, S.S. & Kremen, C. (2006). Wild bees enhance honey bees'pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences,* Vol. 103, No. 37, p. 13890-13895, ISSN 1091-6490

Hagler, J.R. (1990). Honeybee (*Apis mellifera* L.) response to simulated onion nectars containg variable sugar and potassium concentrations. *Apidologie*, Vol.21, p. 115-121, ISSN 12979678

Harbone, J.B. (1998). *Phytochemical methods: a guide to modern techniques of plant analysis,* Chapman & Hall, ISSN 0973-1296, New York, USA

Heinrich, B. (1979). Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia*, Vol. 40, p. 235-245, ISSN 00298549

Herrera, C.M.; Veja, C.; Canto, A. & Pozo, M.I. (2009). Yeasts in floral nectar: a quantitative survey. *Annals of Botany*, Vol. 103, No. 9, pp. 1425-1423, ISSN 1095-8290

James, R.R. & Pitts-Singer, T.L. (2008). The future of agricultural pollination. In: _____ *Bee pollination in Agricultural Ecosystems.* Osford University Press, ISBN 978-0-19-531695-7, New York, USA

Kevan, P.; Lee, H. & Shuel, R.W. (1991). Sugar rations in nectars of varieties of canola (*Brassica napus*). *Journal of Apicultural Research,* Vol. 30, No. 2, p. 99-102, ISSN 0021-8839

Kevan, P. & Phillips, T.P. (2001). The economic impacts of pollinator declines: an approach to assessing the consequences. *Conservation Ecology*, Vol. 5, No.1, p. 1-13, available on line URL: http://www.consecol.org/vol5/iss1/art8/ accessed on Ap 14th 2009.

Klein, A.M.; Vaissière, B.E.; Cane, J.H. & Steffan-Dewenter, I. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society of London: Series B*, Vol. 274, p. 303-313, ISSN 1471-2954

Lanza, J.S.; Smith G.C. & Sack, S. (1995). Variation in nectar volume and composition of *Impatiens capensis* at the individual, plant, and population levels. *Oecologia*, Vol.102, p. 113-119, ISSN 0029-8549

Long, J.E. (1991). High fructose corn syrup. *Food Science and Technology*, Vol. 48, 247-258, ISSN 1226-7708

Malagodi-Braga, K.S. (2005). Abelhas: por quê manejá-las para a polinização? *Revista Mensagem Doce*, No.80, ISSN 1981-6243

Malerbo-Souza, D.T.; Nogueira-Couto, R.H.; Couto L.A. (2003). Polinização em cultura de laranja (*Citrus sinensis* L. Osbeck, var. Pera-rio). *Brazilian Journal of Veterinary Research and Animal Science,* Vol. 40, No. 4, p. 237-242, ISSN 1413-9596

Malerbo-Souza, D.T.; Nogueira-Couto, R.H. & Couto, L.A. (2004). Honey bee attractants and pollination in sweet Orange, *Citrus sinensis* (L.) Osbeck, var. Pera-Rio. *Journal of Venomous Animals and Toxins including Tropical Diseases*, Vol.10, No.2, p. 144-153, ISSN 1678-9199

Martin, E.A.C.; Machado, R.J.P. & Lopes, J. (2005). Atrativo para abelhas em campos de produção de sementes de girassol colorido híbrido. *Semina: Ciências Agrárias*, Vol.26, No.4, p. 489-494, ISSN 1676-546X

Messineo, L. & Musarra, E. (1972). Sensitive spectrophotometric determination of fructose, sucrose, and inulin without interference from aldohexoses, aldopentoses, and ketopentoses. *International Journal of Biochemistry*, Vol.3, No.18, p. 691-699, ISSN 1357-2725

Moerman, F.T.; Van Leeuwen, M.B. & Delcour, J.A. (2004). Enrichment of higher molecular weight fractions in inulin. *Journal of Agricultural and Food Chemistry*, Vol.52, p. 3780-3783, ISSN 0021-8561

Moreira, R.F.A. & De Maria, C.A.B. (2001). Glicídeos no mel. *Química Nova*, Vol. 24, No. 4, p. 516-525, ISSN 1678-7064

Morgado, L.N.; Carvalho, C.F.; Souza, B. & Santana, M.P. (2002). Fauna de abelhas (Hymenoptera: Apoidea) nas flores de girassol *Helianthus annuus* L., em Lavras - MG. *Revista Ciência e Agrotecnologia*, Vol.26, No.6, p. 1167-1177, ISSN 1413-7054

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, Vol.31, No.3, p. 426-428, ISSN 0003-2700

Müller, A.; Diener, S.; Schnyder, S.; Stutz, K.; Sedivy, C.; Dorn, S. (2006). Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee-flower relationships. *Biological Conservation*, Vol.130, No.4, p. 604-615, ISSN 0006-3207

Nicolson, S.W.; Nepi, M. (2005). Dilute nectar in dry atmospheres: nectar secretion patterns in *Aloe castanea* (Asphodelaceae). *International Journal of Plant Sciences,* Vol. 166, No. 2, p. 227-233, ISSN 1058-5893

Paiva, G.J.; Terada, Y. & Toledo, V.A.A. (2002). Behavior of *Apis mellifera* L. Africanized honeybees in sunflower (*Helianthus annuus* L.) and evaluation of *Apis mellifera* L. colony inside covered area of sunflower. *Acta Scientiarum*, Vol.24, No.4, p. 851-855, ISSN 1679-9275

Percival, M. (1953). Types of nectar in angiosperms. *New Phytologist,* Vol. 60, p. 235-281, ISSN 1469-8137.

Pham-Delegue, M.H.; Etievant, P.; Ghuichard, E.; Marilleau, R.; Doualt, P.H.; Chauffaille, J. & Masson, C. (1990). Chemicals involved in honeybee sunflower relationship. *Journal of Chemical Ecology*, Vol.16, No.11, p. 3053-3065, ISSN *0098-0331*

Prince, P.W. (1997). *Insect Ecology,* John Wiley & Sons, New York, ISBN 0471161845

Proctor, M.; Yeo, P. & Lack, A. (1996). *The natural history of pollination*, Harper Collins Publishers, London, ISBN 0002199068

Robacker, D.C.; Flottum, P.K. & Sammataro, D. (1983). Effects of climatic and edaphic factors on soybean flowers and of the subsequent attractiveness of the plants to honey bees. *Field Crops Research*, Vol. 6, No. 4, p. 267-278, ISSN 0378-4290

Roberts, R.B. (1979). Spectrophotometric analysis of sugars produced by plants and harvested by insects. *Journal of Apicultural Research,* Vol. 18, p. 191-195, ISSN 2078-6913

Roe, J.H. (1934). A colorimetric method for the determination of fructose in blood and urine. *Journal of Biological Chemistry*, Vol.107, p. 15-22, ISSN 0021-9258

Roe, J.H.; Epstein, J.H. & Goldstein, N.P. (1949). A photometric method for the determination os inulin in plasma and urine. *Journal of Biological Chemistry*, Vol.178, p. 839-845, ISSN 0021-9258

Ruiz-Matute, A.I.; Weiss, M.; Sammataro, D.; Finely, J. & Sanz, M.L. (2010). Carbohydrate composition of high-fructose corn syrups (HFCS) used for bee feeding: effect on honey composition. *Journal of Agricultural and Food Chemistry,* Vol. 58, p.7317-7322, ISSN 0021-8561

Schirmer, L.R. (1985). *Abelhas ecológicas*, Nobel, São Paulo: Nobel, ISBN 9253012536

Severson, D.W. & Erickson, J.E.H. (1984). Quantitative and qualitative variation in floral nectar of soybean cultivars in southeastern Missouri. *Environmental Entomology*, Vol. 13, No. 4, pp. 91-96, ISSN 1938-2936

Shafir, S.; Bechar, A.; Weber, E.U. (2003). Cognition mediated coevolution: context-dependent evaluations and sensitivity of pollinators to variability in nectar rewards. *Plant Systematic and Evolution,* Vol. 238, No. 1-4, p. 195-209, ISSN 1615-6110

Sheppard, W.S.; Jaycox, E.R. & Parise, S.G. (1978). Selection and management of honey bees for pollination of soybean. In: International Symposium of Pollination, 4., University of Maryland College Park, Maryland, USA. *Proceedings…* 1, p. 123-130

Siddiqui, I.R. (1970). The sugars of honey. *Advances in Carbohydrate Chemistry and Biochemistry,* Vol. 7, p. 51-59, ISSN 0065-2318

Silva, E.M.; Dean, B.B. (2000). Effect of nectar composition and nectar concentration on honey bee (Hymenoptera: Apidae) visitations to hybrid onion flowers. *Journal of Economic Entomology*, Vol. 93, No. 4, p. 1216-1221, ISSN 0022-0493

Sumner, D.A. & Boriss, H. (2006). Bee-conomics and the leap in pollination fees. *Giannini Foundation of Agricultural Economics,* Vol. 9, p. 9-11, ISSN 1081-6526

Summer, J.B. & Sisler, E.B. (1944). A simple method for blood sugar. *Archives of Biochemistry and Biophysics*, Vol.4, p. 333-336, ISSN 0003-9861

Täufel, K. & Reiss, R. (1952). Analytische und chromatographische Studien am Bienenhonig. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, Vol. 94, No. 1, p. 1-10, ISSN 0044-3026

Teixeira, L.M.R. & Zampieron, S.L.M. (2008). Estudo da fenologia, biologia floral do girasol (*Helianthus annuus*, Compositae) e visitantes florais associados, em diferentes estações do ano. *Ciência et Praxis*, Vol.1, No.1, p. 5-14, ISSN 1983192X

Thomson, J.D. & Goodell, K. (2002). Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology*, Vol.38, No.5, p. 1032-1044, ISSN 365-2664

Toledo, V.A.A.; Oliveira, A.J.B.; Ruvolo-Takasusuki, M.C.C.; Mitsui, M.H.; Vieira, R.E.; Kotaka, C.S.; Chiari, W.C.; Gobbi Filho, L.; Terada, Y. (2005). Sugar content in nectar flowers of siratro (Macroptilium atropurpureum Urb.) *Acta Scientiarum – Animal Science,* Vol. 27, No. 1, p. 105-108, ISSN 1807-8672

Vidal, M.D.G.; De Jong, D.; Wien, H.C. & Morse, A.R. (2006). Nectar and pollen production in pumpkin (*Cucurbita pepo* L.). *Revista Brasileira de Botânica*, Vol.29, No.2, p. 267-273, ISSN 0100-8404

Vidal, M.D.G.; De Jong, D.; Wien, H.C. & Morse, A.R. (2010). Pollination and fruit set in pumpkin (*Cucurbita pepo*) by honey bees. *Revista Brasileira de Botânica*, Vol.33, No.1, p. 106-113, ISSN 0100-8404

Vieira, R.E.; Kotaka, C.S.; Mitsui, M.H.; Taniguchi, A.P.; Toledo, V.A.A.; Ruvolo-Takasusuki, M.C.C.; Terada, Y.; Sofia, S.H.; Costa, F.M. (2002). Biologia floral e polinização por abelhas em siratro (*Macroptilium atropurpureum* Urb.). *Acta Scientiarum*, Vol. 24, No. 4, p. 857-861, ISSN 1806-2636

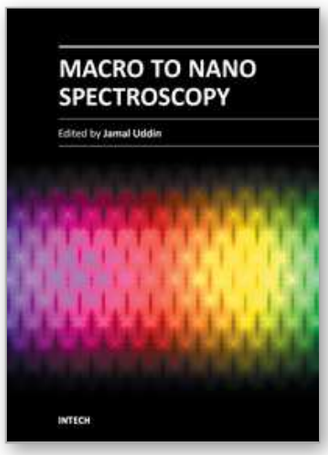
Waller, G.D. (1972). Evaluating responses of honeybees to sugar solutions using an artificial-flower feeder. *Annals of the Entomological Society of America*, Vol. 65, p. 857-862, ISSN 0013-8746

Wykes, G. R. (1952a). An investigation of the sugars present in the nectar of flowers of various species. *New Phytologist*, Vol. 51, No. 2, p. 210-215, ISSN 1469-8137

Wykes, G.R. (1953). The sugar content of nectars. *Biochemical Journal,* Vol. 53, p. 294-296, ISSN 0264-6021

Yemm, E.W. & Willis, J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Journal of Biochemical*, Vol.57, No.3, p. 508-514, ISSN 0264-6021

Zimmerman, M. (1988). Nectar production, flowering phenology, and strategies for pollination. In: Doust, J.L. & Doust, L.L. (Eds.). *Plant reproductive ecology, patterns and strategies*. Oxford, ISBN 01-950-51750, New York, USA



Macro To Nano Spectroscopy
Edited by Dr. Jamal Uddin

ISBN 978-953-51-0664-7
Hard cover, 448 pages
Publisher InTech
Published online 29, June, 2012
Published in print edition June, 2012

In the last few decades, Spectroscopy and its application dramatically diverted science in the direction of brand new era. This book reports on recent progress in spectroscopic technologies, theory and applications of advanced spectroscopy. In this book, we (INTECH publisher, editor and authors) have invested a lot of effort to include 20 most advanced spectroscopy chapters. We would like to invite all spectroscopy scientists to read and share the knowledge and contents of this book. The textbook is written by international scientists with expertise in Chemistry, Biochemistry, Physics, Biology and Nanotechnology many of which are active in research. We hope that the textbook will enhance the knowledge of scientists in the complexities of some spectroscopic approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of chemistry, physics and material sciences.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen