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The Relationship between Fructose, Glucose and Maltose Content with Diastase Number and Anti-Pseudomonal Activity of Natural Honey Combined with Potato Starch

Ahmed Moussa[1]*, Djebli Noureddine[2], Aissat Saad[1] and Salima Douichene[2]

[1]*Institute of Veterinary Sciences University Ibn-Khaldoun, Tiaret, Algeria*
[2]*Departments of Biology, Faculty of Sciences, Mostaganem University, Algeria*

Abstract

Honey whose medicinal uses date from ancient times has been lately rediscovered as therapy for burns.

Objective: To evaluate the additive action of potato starch on the antipseudomonal activity of natural honey.

Methods: Physicochemical parameters of 6 samples of Algerian honeys were analysed; four parameters were measured, including Diastase, glucose, fructose and maltose. The antibacterial activity was tested using the well-agar diffusion assay.

Results: Six honey samples with initial diastase activity between 22.1 and 7.3 Schade units were tested. Glucose, fructose and maltose values range between 21, 45-30, 95%, 25, 20-37, 81% and 4, 72-78, 45% respectively. The zone inhibition diameter (ZID) for the six honey samples without starch against *P. aureogenosa* ranged between 26 and 31 mm. When starch was mixed with honey and then added to well, a zone inhibition increase diameter (ZIID) 27 and 32 mm. The percentage increase (PI %) was noticed with each variety and it ranged between 3, 57 and 18, 75%. Positive correlation has been established between the zone increase of inhibition and the Diastase number (*r* value was 0.072 at $p<0.05$).

Conclusion: The use of potato starch allows honey benefit and would constitute an additive effect to the antibacterial activity of natural honey.

Keywords: Diastase number; Honey; Antibacterial activity; Potato starch

Introduction

Many burn infections are treated with antibiotics that can be applied topically or administered orally or by injection. Unfortunately, due to the excessive use of antibiotics, some bacteria have evolved to become antibiotic resistant, and this has led to the present time being described as the "end of the antibiotic era" [1,2]. The dominant flora of burn wounds during hospitalization changes from Gram-positive bacteria such as *Staphylococcus* to Gram-negative bacteria like *Pseudomonas aeruginosa.* The majority of *P. aeruginosa*, an opportunistic human pathogen, isolates from burn patients were multidrug resistant (MDR) [3-5]. In wounds, *P. aeruginosa* has emerged as a multidrug-resistant organism that gives rise to persistent infections in burns patients [6,7] and chronic venous leg ulcers [8]. Novel antimicrobial interventions are needed.

The complexity of natural products, including honey, makes them very difficult to standardize and this can affect their acceptance in clinical medicine. However, this complexity also has benefits. Unlike conventional antibiotics it appears to be difficult for microorganisms to become resistant to the effects of honey, probably due to the action of the various active components in honey on multiple microbial targets [9]. Honey is the most famous rediscovered remedy that has been used to promote wound and burn healing and also to treat infected wounds [10]. The use of honey in modern clinical practice is based on its broad antimicrobial properties and its ability to stimulate rapid wound healing.

Several bioactive compounds have been identified in honey which contributed to its antibacterial action. The commonly accepted list of contributors includes osmolarity [11]. High osmolarity has been considered a valuable tool in the treatment of infections, because it prevents the growth of bacteria and encourages healing [12]. Honey is a supersaturated sugar solution; and sugar content accounts for more than 95% of the dry matter. Honey is an extremely varying and complex mixture of sugars and other minor components. Fructose is the most dominant sugar followed by glucose in almost all types of honey [13]. Maltose content in natural honey is generally less than 30 mg/g [14,15]. Maltose in some honeys originating from certain plants can be up to 50 mg/g [16,17].

Honey contains small amounts of different enzymes, notably, diastase (α-and β -amylase), invertase (α-glucosidase), glucose-oxidase, catalase and acid phosphatase, which come from nectar sources, salivary fluids and the pharyngeal gland secretions of the honeybee [18]. A diastase is any one of a group of enzymes that catalyze the breakdown of starch into maltose [19]. Alpha amylase degrades starch to a mixture of the disaccharide maltose, the trisaccharide maltotriose and oligosaccharides known as dextrin's [20]. Diastase activity is expressed as the diastase number (DN) in Schade units and is defined as follows: one diastase unit corresponds to the enzyme activity of 1 g of honey,

***Corresponding author:** Ahmed Moussa, Institute of Veterinary Sciences University Ibn-Khaldoun, Tiaret, Algeria, Tel: +213 65234059; Fax: 213 65234059; E-mail: moussa7014@yahoo.fr

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which can hydrolyse 0.01 g of starch in 1 h at 40°C. The range permitted for diastase number varies from 3 to 8 on Gothe's scale, depending on the climate prevailing in the place where the honey originates [21]. In previous studies, we have shown that there is an Additive action between honey and ginger starch in terms of antibacterial [22] and antifungal activity [23,24]. We suggested that α amylases present in honey originating from bees and pollen are responsible in the hydrolysis of starch chains to randomly produce dextrin and maltose that increase the osmotic effect of honey and consequently increase the antibacterial activity. But no starch is found in honey. The aim of this study is to evaluate the potential antibacterial activity of honey and potato starch when used jointly to manage superficial burn.

Materials and Methods

Preparation of honey sample

Six unifloral and multifloral honey samples were collected from beekeepers in different regions of the western Algeria during different seasons of the 2011 year depending on floral sources: Jujube, Citrus, Eucalyptus and Multifloral. All samples were collected in their original packages and were transferred to the laboratory and kept at 4-5°C until analysis. Honey was used within a few hours of preparation to avoid self-decomposition and decrease in diastase activity.

Preparation of the stock starch solution

The stock starch solution was prepared by dissolving 0.5 g of dried soluble starch in deionised water in a volumetric flask. After heating and stirring the solution for approximately ten minutes, starch was completely dissolved, and the volumetric flask was filled with deionised water to the mark.

Physicochemical analyses

All physicochemical tests were performed in triplicate.

Determination of maltose, glucose and fructose contents

Sugar spectra (fructose, glucose, and maltose) were identified and determined by Bogdanov [25] for di- and oligosaccharides using high-performance liquid chromatography (HPLC).

Diastase activity (Diastase number)

Diastase activity was measured with Phadebas, according to the Harmonized Methods of the European Commission of Honey [25]. An insoluble blue dyed cross-linked type of starch is used as the substrate. This is hydrolysed by the enzyme, yielding blue water-soluble fragments, determined photometrically at 620 nm. The absorbance of the solution is directly proportional to the diastatic activity of the sample. The diastase activity, expressed as DN or diastase number, was calculated from the absorbance measurements using Eqs. (1) and (2) for high (8–40 diastase units) and low (up to 8 diastase units) activity values, respectively:

$$DN = 28.2 \times \Delta A620 - 2.64 \quad (1)$$

$$DN = 35.2 \times \Delta A620 - 0.46 \quad (2)$$

Bacterial culture and inoculum preparation

Pure culture of *P. aeruginosa* ATCC 27853 was obtained from the Department of Biology, Faculty of Sciences, Mostaganem University, Algeria. The bacteria was grown on Nutrient Agar (NA; Merck Germany) slant, incubated at 37°C for 24 h, and kept at 4°C until further use. Bacterial suspension was prepared by inoculating one loopful of the 24-h-old bacterial colonies into 10.0 ml of sterilized distilled water. The inoculums size was adjusted to match the turbidity of McFarland 0.5 scale (1×10^8 cells/ml) and diluted with sterilized distilled water to the inoculums size of 1×10^7 cells/ml.

Measurement of zone of inhibition (Well diffusion assay)

A screening assay using well diffusion [26] was carried out with some minor modifications. Nutrient agar plates (Merck, Germany) were inoculated by rubbing sterile cotton swabs that were dipped into bacterial suspensions (over night) cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate. After inoculation 8.2 mm diameter wells were cut into the surface of the agar using a sterile cork borer. 50 µl of test honey was added to each well. Plates were incubated at 30°C for 24 h. A diffusion control of starch was used. Second step a mixture of starch-honey was prepared and incubated for one hour at 40°C. After inoculation 8.2 mm diameter wells were cut into the surface of the agar using a sterile cork borer. 50 µl of mixture (honey and starch) were added to each well. Zones of inhibition were measured using a Vernier caliper. The diameter of zones, including the diameter of the well, was recorded. Bioassay was performed in duplicate and repeated twice. The results were expressed in terms of the diameter of the inhibition zones:<5.5 mm, inactive; 5.5-9 mm, very low activity; 9-12 mm, low activity; 12-15 mm, average activity; and >15 mm, high activity.

Statistical analysis

Each honey was analyzed in triplicate. Results are shown as mean values and standard deviation. Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($p<0.01$). All statistical analyses were performed with the Statistica 7.0 software for Windows.

Results and Discussion

Physicochemical parameters

Table 1 reports the physico-chemical parameters of the honey

Honey samples	glucose (g/100 g honey)		fructose (g/100 g honey)		maltose (g/100 g honey)		Sugar total content (%)		Diastase activity (Schade Number[b])	
	Mean	DS	Mean	DS	Mean	DS	Mean	DS	Mean	DS
H1	21.45	2.14	25.20	3.32	0.00	1.32	46.65	2.26	15.1	2.8
H2	26.18	2.14	37.81	3.32	7.13	1.32	71.12	2.26	23.5	2.8
H3	25.78	2.14	33.92	3.32	8.45	1.32	68.45	2.26	11	2.8
H4	28.84	2.14	36.84	3.32	7.01	1.32	72.69	2.26	26	2.8
H5	27.24	2.14	35.41	3.32	4.72	1.32	67.37	2.26	7.3	2.8
H6	30.95	2.14	35.18	3.32	7.10	1.32	73.23	2.26	16.4	2.8

[b]Schade number. corresponds with Gothe number, or 0.01 g starch hydrolysed 1h at 40°C per 1 g honey.

Table 1: The concentration of glucose, fructose and maltose in the honey samples (g/ 100 g) and diastase activity results represent the average of four measurements ± SD (n=3).

samples. The mean, standard deviation (SD) and the variable ranges are reported for comparison with international standards. Fructose, glucose and maltose values range between 25, 20-37, 81%, 21, 45-30, 95% and 4, 72-78, 45%, respectively.

Analysis of amylase activity

The diastatic activity in honey is considered a quality factor. It decreases during storage, heat treatment and feeding of honeybees during honey flow; thus, it is an indicator of honey ageing, adulteration and overheating. The honey samples analyzed in the present work show a range of values, between 22.1 and 7.3 Schade units. One sample (H5) show values below 8 Schade units (Table 1). The explanation for the low content of diastatic activity found in this one sample could be accounted for an inadequate processing or storage conditions.

Antibacterial activity

The six honey samples were studied in terms of antibacterial activity were performed in duplicate. (Table 2) and (Figures 1 and 2) summarize the zones of inhibition of the honey samples against the tested organism. The differences in inhibition were observed for six types of honey sample (H5) has the largest inhibition with an average diameter of 31 mm, followed by the sample (H6) in (30 mm), H4 (29 mm), H2 (28 mm), H3 (27 mm), and finally the sample H1 (26 mm). No zone of inhibition was determined with starch alone.

The differences in inhibition were observed for six types of honey

Honeysample	Honey only	Starch and honey %(v/v)	Zone increase of inhibition Percentage increase (PI%)
H1	26	32	18.75
H2	28	28	3.57
H3	27	27	3.57
H4	29	30	3.57
H5	31	31	00
H6	30	30	00

<5.5 mm. inactive; 5.5–9 mm. very low activity; 9–12 mm. low activity; 12–15 mm. average activity; and >15 mm, high activity

Table 2: Mean Zones of Inhibition (diameter mm including well (8.2 mm).

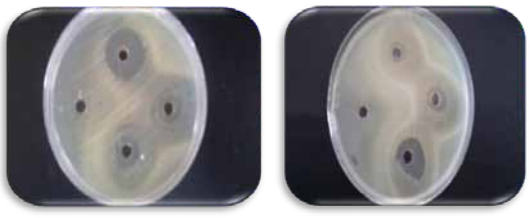

Figure 1: Inhibition Zone Diameters of natural honey only against *P.aeruginosa*.

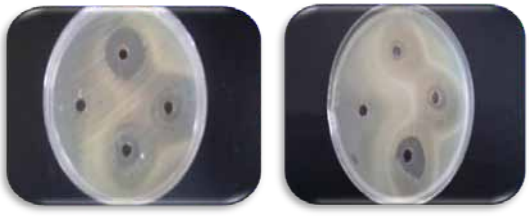

Figure 2: Inhibition Zone Diameters of natural honey with potato starch against *P. aeruginosa*.

with starch potato: the sample (H5) has the largest inhibition with an average diameter of 31 mm, followed by the sample (H1) in (31 mm), H5 (31 mm), H4 and H6) (30 mm), H2 (28 mm) and finally the sample H3 (27 mm). The percentage increase (PI%) was noticed with each variety and it ranged between 3, 57 and 18, 75%.

Pseudomonas aeruginosa is the predominant cause of fatal burn wound sepsis, and isolation of multidrug-resistant strains is a common problem in hospitals. With increasing interest in the use of alternative therapies and as the development of antibiotic resistant bacteria spreads. Many works was interested, during this last decade, with the products of the hive and in particular honey, efficient product against the germs secreted by the bees as a possible source of new pharmaceutical and medical agent. In Algeria, there are few types of alternative medicine such as honey. Recent experimental finding indicated that the amylase present in honey increases the osmotic effect in the media by increasing the amount of sugars and consequently increasing the antibacterial activity [22]. High osmolarity has been considered a valuable tool in the treatment of infections, because it prevents the growth of bacteria and encourages healing [27]. Use of sugar to enhance wound healing has been reported for several hundred patients [28].

Molan [29] has studied sugar syrups of the same water activity as honey and found them to be less effective than honey at inhibiting microbial growth *in vitro*. It was found that solutions of high osmolarity, such as honey, glucose, and sugar pastes, inhibit microbial growth because the sugar molecules tie up water molecules so that bacteria have insufficient water to grow [30]. Therefore, high osmolarity is valuable in the treatment of infections because it prevents the growth of bacteria and encourages healing. Sugar was used to enhance wound healing for several hundred patients [31]. It has been claimed that the sugar content of honey is responsible for its antibacterial activity, which is due entirely to the osmotic effect of its high sugar content [32].

In our study, the addition of starch potato honey showed a significant increase in the inhibition zone for honey studied against the strain tested except honey H5 and H6. Amylase present in honey was expected to split potato starch chains into randomly produced dextrin and maltose and probably increases the osmotic effect in the well by increasing the amount of sugar and consequently increases the antibacterial activity. Other results show that the addition ginger starch to honey could contribute to reducing the quantity of honey to be used without losing the expected effect [22-24]. In our study, the amount of the amylase present in honey is in positive correlation with the relative potency of starch and honey.

Neither honey nor starch has adverse effects on tissues, so they can be safely used in wounds, burns and inserted in cavities and sinuses to clear infection. A clinical trial would be carried out to validate these findings. The results will enable a systematic study of many varieties of honey on pathogens bacteria withincreased resistance opposite conventional antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

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