ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Honey, Probiotics and Prebiotics: Review

Bemmo Kamdem Ulrich Landry[1], Sahoo Moumita[2], R Jayabalan[2*], and Zambou Ngoufack François[1#].

[1]Laboratory of Biochemistry, Food Science and Nutrition (LABPMAN) - Department of Biochemistry - Faculty of Science - University of Dschang, P.O. Box 67 Dschang, Cameroon.
[2]Food Microbiology and Bioprocess Laboratory, Department of Life Science, National Institute of Technology, Rourkela 769 008, Odisha, India.

ABSTRACT

Nowadays, health awareness has risen to a great extent and hence non-dairy probiotic products are in high demand by consumers due to the major set-backs, allergy, and lactose intolerance, of the dairy based probiotic counterparts being commercialized. The functional attributes of such non-dairy probiotic products can be enhanced many fold by coalescing it with some natural prebiotic oligosaccharides. Prebiotics are substances which cannot be digested by pathogenic microorganisms and thereby selectively enhance the development of probiotics. Probiotics are live microorganisms which confer health-benefits in humans. Keeping this in view, the present review focuses on honey being an exceptional source of such prebiotic oligosaccharides (fructo-oligosaccharide, galacto-oligosaccharide) perfect for utilization to produce non-dairy synbiotic formulations. Honey is a natural food consumed by humans since many centuries by almost all civilizations due to its rich nutritional and therapeutic values. It remains stable at room temperature for long time. The complex nature of honey does not favor the development of majority of microorganisms. However, honey has been reported to stimulate the growth and/or activity of probiotic microorganisms due to its prebiotic content.

Keywords: Honey, Probiotic, Prebiotic, Synbiotic, Oligosaccharides

Corresponding author, #Co-corresponding author

ISSN: 0975-8585

INTRODUCTION

Dairy products have long been used as carriers for probiotic bacteria by humans. However, these dairy products contain lactose, allergen compounds, and cholesterol and they require specific equipment for cold storage due to their perishable nature. Due to all these factors, the need of new non-dairy based probiotic product has become a major objective of many researchers [1, 2]. The development of new non-dairy probiotic food products is very much challenging as it has to meet the consumer's expectancy for health benefits [3]. In addition to being the most ancient food consumed by human, honey is a natural sugar produced by honeybees. Honey is rich in simple sugars and contains small quantity of oligosaccharides [4]. Shin and Ustunol (2005) have shown that honey sugars can stimulate the growth of bifidobacteria [5].

HONEY

Honey is natural sweet substance produced by honeybees from the nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform, and combine with specific substances of their own, store, and leave in the honey comb to ripen and mature [6]. Hence, honey is subdivided into two categories: "nectar honey" or blossom honey and "honeydew honey" or forest honey [7]. Mono-floral honeys are made from nectar and/or honeydew from a single plant species and this requires installing beehives near the plant sought. The poly-floral honeys are made from nectar and/or honeydew from several plant species. Nectar Honey comes from nectars of plants. Nectar is a sweet exudation more or less viscous depending on its water content. This nectar is produced by the nectar glands from the sap of the plant. Honeydew honey comes mainly from excretions of plant sucking insects (*Hemiptera*) on the living parts of plants or secretions of living parts of plants [6]. The composition of honey and its properties are dependent on the botanical origin of nectar.

COMPOSITION OF HONEY [4, 7, 8]

Honey is reported to have different compositions with varying concentrations of carbohydrates, organic acids, proteins, amino acids, vitamins, and flavor components in water medium. Detailed composition of honey is listed in Table 1.

Table 1: Composition of honey (g/100g)

	Blossom honey		Honeydew honey	
	Average	Min-max	Average	Min-max
Water content	17.2	15-20	16.3	15-20
Fructose	38.2	30-45	31.8	28-40
Glucose	31.3	24-40	26.1	19-32
Sucrose	0.7	0.1-4.8	0.5	0.1-4.7
Melezitose	<0.1		4.0	0.3-22.0
Erlose	0.8	0.56	1.0	0.16
Total sugars	79.7		80.5	
Minerals	0.2	0.1-0.5	0.9	0.6-2
Amino acids, proteins	0.3	0.2-0.4	0.6	0.4-0.7
Acids	0.5	0.2-0.8	1.1	0.8-1.5
pH	3.9	3.5-4.5	5.2	4.5-6.5

Carbohydrates

Honey is composed of about 80% carbohydrates. Among the carbohydrates, we mainly find fructose and glucose which are monosaccharides. Small amounts of polysaccharides such as sucrose, melezitose, and other sugars are also present. In total, over 25 different polysaccharides are reported in honey. The sugar spectrum varies according to the kind of honey: the content of glucose and fructose is specific to the kind.

Water

In honey, the water content depends on the nectar flow, climate, type of hive, and other factors. It is usually less than 20% and then determines predominantly the keeping of honey. When honey has water content less than 17%, it is more stable during storage and do not ferment.

Acids

Honey contains less than 1% (forest honey <2%) organic acids, nevertheless, these acids play a decisive role in the formation of its taste. The rich acid honeys (eg. honeydew) seem less sweet than those containing fewer acids (eg. acacia honey). The main acid present is gluconic acid. Other acids such as formic acid, lactic acid, and oxalic acid are also found. Most honeys are acidic i.e., their pH is less than 7.0.

Amino acids and proteins

Honey proteins consist mainly of enzymes from the secretions of bees. The amino acids present in honey come on the one hand from the nectar flow and on the other hand from bee secretions. Proline is the main amino acid from the bees.

Hydroxymethylfurfural

The hydroxymethylfurfural (HMF), sugar degradation product, appears only after honey collection. According to the Swiss Food Manual, fresh honey, natural, can contain only 15 mg HMF / kg, while for other retail honey, 30 mg / kg or less is permitted.

Minerals and Vitamins

Honey contains various minerals and vitamins. Honeydew honeys are richer in minerals than nectar honey. Potassium is the main mineral present. Mineral content in blossom honeys is between 0.1 - 0.3% and can reach 1% in honeydew honeys. Honey contains a number of minerals and trace elements (Table 2).

Table 2: Trace elements present in honey

Element	mg/100g	Element	mg/100g
Aluminium (Al)	0.01-2.4	**Fluoride (F)**	0.4-1.34
Arsenic (As)	0.014-0.026	Iodine (I)	10-100
Barium (Ba)	0.01-0.08	Lithium (Li)	0.225-1.56
Boron (B)	0.05-0.3	Molybdenum (Mo)	0-0.004
Bromine (Br)	0.4-1.3	Nickel (Ni)	0-0.051
Chlorine (Cl)	0.4-56	Rubidium (Rb)	0.040-3.5
Cobalt (Co)	0.1-0.35	Silicium (Si)	0.05-24

Flavor and flavonoids

We can characterize 500 different aromatic substances in honey. Although the latter are present only in trace, they nevertheless play a key role in the formation of the taste of honey. When the honey is heated, a part of them are lost. As for the flavonoids, they are partially responsible for the color of honey. Dark colored honey contains more phenolic acid derivatives but less flavonoids than the lighter ones [7].

Microorganisms

Honey has a high concentration of sugars and therefore a high osmotic pressure that cannot favor the development of microorganisms. In honey much less bacteria is found than in other raw products of animal origin. No pathogenic *Bacillus* species have been detected in honey. There are cases of infants poisoning due to honey contaminated with *Clostridium botulinum* bacteria.

PROPERTIES OF HONEY

Honey has a very high nutritional as well as therapeutic value known since ancient times.

Nutritional and dietary values

Honey, food of all ages, has stayed until the last century, the sweetener of choice for food, until the arrival of cane sugar. Because of its high concentration of sugar, honey is an excellent source of energy. Honey represents an energy input of about 300 kcal per 100 g. Sugars in honey are quickly absorbed. Furthermore, honey has sweetness greater than sucrose. Indeed, the sweetening power of fructose and glucose is 1.3 compared to 1 for sucrose from sugar cane or beets [8]. In young children, honey also promotes calcium absorption and retention of magnesium in the body contributing to better bone and dental calcification. Enzymes, vitamins, and trace elements are also present in honey which facilitates digestion. However, there is a risk of botulism in children under 1 year if the honey consumed is contaminated with spores of *Clostridium botulinum*. It will be best to avoid the consumption of honey before 1 year or, it is advisable to consume pasteurized honey [9]. For a variety of reasons such as poor appetite, indigestion, disturbed bowel function, loss of energy, fatigue, vitamin deficiencies, and supply of trace elements, etc., regular consumption of honey is advisable. It could help older people to overcome deficits, in particular by increasing their appetite. Honey is also reported to have a protective effect against irritation of the digestive mucosa that appears with age. Honey acts by promoting a relative rest of the intestines because most of its components are absorbed as such without prior processing. Moreover, very few food components provide a natural concentration of fructose as high as honey. This feature partially explains its "laxative" power which is an advantage for individuals with physical inactivity which disrupts intestinal function. Due to its relatively large calorific value, honey is a food of choice for athletes. In the short term, glucose is an excellent fuel immediately available; at longer term, fructose takes over, because it is indeed captured by the liver (without the intervention of insulin) and kept in reserve as glycogen [8]. Honey is often considered a "functional food" that is to say a food ready for therapeutic virtues. Indeed, it is credited with many therapeutic and pharmacological properties.

Therapeutic and pharmacological properties of honey

Besides their nutritional qualities, all honeys have common therapeutic and pharmacological properties more or less marked according to their floral origin. With the increasing prevalence of antibiotic-resistant bacteria, honey is increasingly valued for its antibacterial activity. Currently, five main factors are responsible for the antibacterial activity of honey: osmolarity, acidity, hydrogen peroxide system (inhibin), phytochemical factor bee defensin-1, and methylglyoxal (mgo) [10]. It is also reported to have healing properties, expectorant, anti-cough, action against chronic constipation, liver detoxification, role in alleviating digestive disorders (protection of the wall of the stomach, treatment of peptic ulcers) [8].

With all these interesting properties of honey on the health of consumers, the demand for honey is rapidly increasing day by day. Thus, to satisfy this demand profitably, producers and sellers develop fraudulent techniques (sugar addition, mix of two different honeys, etc.) to increase honey production. Therefore, to meet these maneuvers, there is an urgent need to focus on controlling the quality of honey in the market.

The quality criteria of honey

The main quality parameters are: color, moisture, pH and acidity, spectrum of sugars, the content of 5-hydroxy-2-methylfurfural (HMF), the activity of amylase (diastase), and the activity of invertase.

pH and acidity

Honey contains a wide range of acids which come directly from nectar (or honeydew), or from other enzyme reactions, and fermentations. The study of the acidity of honey helps to identify its botanical origin. Nectar honeys have a low pH 3.3 to 4.5, while honeydew honeys have a slightly higher pH (4.5 to 5.5). An extreme pH values out of these intervals reveal a biochemical degradation of honey due to poor harvesting conditions or conservation [4, 11]. Free acidity is expressed as milliequivalents (mEq) of sodium hydroxide required to neutralize 1 kg of honey and should not exceed 50 **meq/kg** [6].

Color

The color of a honey can be modified by the following factors: botanical origin, age, and storage conditions. Honey has various colors: from colorless to black, with beige, yellow, orange, and brown [11, 12]. Phenolic acid derivatives also have a role to play in the color of honey.

Moisture

Legally, the honey must not exceed 20% moisture [13]. Above this threshold, honey will ferment quickly or will crystallize poorly. The moisture level of honey is measurable with a refractometer.

Spectrum of sugars

The quality of honey depends on one hand on the total glucose and fructose content and on the other hand on the total sucrose content. Other sugars such as isomaltose, melezitose, trehalose, raffinose, palatinose, melibiose, nigerose, turanose, kojibiose, etc. can be detected by chromatographic or spectroscopic methods [14]. Although the amount and type of sugars are very much influenced by the enzymes of the bee, the spectrum of sugars of honey can also give details of the nature of the source plants [7]. The spectrum of sugars has its importance in fraud detection.

Invertase activity

The freshness of honey can also be determined by the activity of invertase. This enzyme is sensitive to heat and storage [12].

Diastase activity

The diastase activity of honey determined after processing and/or blending is generally not less than 8 Schade units and those with low natural enzyme content should not be less than 3 Schade units [6].

Hydroxy Methyl Furfural (HMF)

Ingestion of a high concentration of honey HMF (40 or 80 **mg/kg**) does not present any risks but it is a mark of denatured honey. Thus, analysis of the amount of HMF is an excellent method to assess the quality of a honey [15].

Honey is a natural food whose health benefits are well established. However, any combination of honey and probiotics could give additional health benefits.

PROBIOTICS

The term probiotic is derived from two Greek words, pro and bios, which literally means "for life".

History and definition

The term probiotic was used for the first time by Elie Metchnikoff in the last century, a Russian researcher. He observed the positive role played by certain bacteria on health. Metchnikoff suggested that "the dependence of intestinal microbes by diet makes possible the adoption of measures to change the body microflora by replacing harmful microbes by useful microbes" [16]. It was popularized by Fuller in 1989 when he gave his first official definition: "Probiotics are dietary supplements containing live microorganisms which have beneficial effects on human being by improving balance and the stability of their intestinal microflora". This definition was revised several times and currently the most accepted is the one recommended by a panel of experts appointed by the United Nations Food and Agriculture Organization and the World Health Organization. It indicates that probiotics are "live microorganisms which, when administered in adequate amounts, confer health benefits on the host" [17]. Moreover, even if it is not mentioned in this definition, the generally recommended dose to achieve beneficial effects is around 10^9 to 10^{10} CFU/ml per day in order to obtain about 10^8 live bacterial cells at the level of duodenum [18]. The contribution must be regular since they

do not colonize the intestine permanently [19]. This may be due to the fact that the resident microbiota composition is specific to each individual and that exogenous bacteria are not easily established [20]. The probiotic designation assigned to a microorganism is not random.

Selection criteria for probiotics [17]

The Joint Committee FAO/WHO established criteria and a methodology for the evaluation of probiotics and also defined the necessary data to the justification of health claims. Therefore potentially probiotic microorganisms must meet several criteria:

Safety Criteria

Microorganisms must have a history of non-pathogenicity, which means they should be generally recognized as safe (GRAS). They must also be of human or food origin, characterized by phenotypic and genotypic methods, be deposited in an international culture collection, present no possibility of transmission of antibiotic resistance genes and not be responsible for the dehydroxylation of bile salts.

Technological criteria

The strain should also be stable during the production process and in the finished product, and its probiotic properties should also be preserved after production.

Functional criteria

After the safety and technological criteria, the strain must show tolerance to gastric acidity and bile salts, be antagonist towards pathogens and produce antimicrobial substances (bacteriocins), adhere to various intestinal cell lines and/or mucus and can boost the immune system. Probiotics should then also show some positive effects on the health of human.

Probiotic health benefits (Table 3)

The probiotic lactic acid bacteria improve digestion and nutrient absorption by producing digestive enzymes [21]. In the same way, the production of organic acids [22], hydrogen peroxide [23], and bacteriocins [24] by probiotics inhibit the pathogenic microorganisms. *Lactobacillus* strains have been reported to improve acute rotavirus diarrhea in children [25, 26]. The consumption of probiotics is a new therapeutic strategy in preventing or delaying the onset of diabetes and subsequently reducing the incidence of hypertension. The concentration of *Bifidobacterium spp.* in the gut was positively correlated with improved glucose tolerance and glucose-induced insulin-secretion as well as decreased diabetes endotoxemia, plasma, and adipose tissue inflammatory cytokines [27]. Several studies have also shown that bifidobacteria can reduce the intestinal endotoxin levels and improve mucosal barrier thus reducing systemic inflammation and subsequently reducing the incidence of diabetes [28]. Probiotic strains can also reduce allergy [29, 30]. The precise mechanisms have not been elucidated, but it relies on the ability of lactobacilli to neutralize the increased intestinal permeability, strengthen response specific IgA in bowel, promote the gut barrier function by restoring normal microbes, and enhance the transformation of the growth factor beta and the production of interleukin 10 and cytokines that promote the production of IgE antibodies [31, 32]. Ong and Shah [33] examined the release of ACE inhibitory peptides in Cheddar cheese made with starter lactococci and probiotics. ACE-inhibitory peptides have also been found in yogurt, cheese, and milk fermented with *L. casei* ssp. *rhamnosus*, *L. acidophilus,* and bifidobacteria strains [34]. The study of Pawan and Bhatia showed significantly enhanced immune response by probiotic consumption and non-significant reduction in cholesterol level, HDL-C in the human subjects and decrease in systolic blood pressure in hypertensive patients by the consumption of *'Dahi'* and *'Lassi'* was also observed [35]. The results of Kumar suggested that the indigenous *L. plantarum* Lp91 strain has the potential to be explored as a probiotic in the management of hypercholesterolaemia [36]. The consumption of fermented milk containing an appropriate strain of *L. acidophilus* has the possibility to reduce the risk of coronary heart disease by 6 to 10%. Every 1% reduction of cholesterol level in the blood is associated with the reduction of the coronary heart disease between 2% to 3% [37]. Many other authors demonstrated the hypocholesterolemic effects of probiotic lactic acid bacteria [22, 24, 38, 39]. Probiotics reduce cholesterol levels by several mechanisms: (a) Deconjugation of bile salts through the bile salt hydrolase enzyme [40, 41];

(b) reduction of the synthesis of apolipoprotein B-100 [42]; (c) inhibition of HMG-CoA reductase by certain metabolites of lactobacilli [43]; (d) ability to assimilate and fix the cholesterol in their cell walls [36, 44]. The growth and activities of probiotics are generally enhanced by substances called prebiotics [45, 46].

Table 3: Health benefits of probiotic bacteria to the host, and postulated mechanisms involved [45,46]

Health benefits	Proposed mechanisms involved
Aid in lactose digestion	✓ Bacterial lactase hydrolyses lactose
Resistance to enteric pathogens	✓ Antagonism activity ✓ Adherence to intestinal mucosa, interfering with pathogen adherence ✓ Limiting access of enteric pathogens (organic acids, bacteriocins/defensins, toxic oxygen metabolites) ✓ Upregulation of intestinal mucin production, interfering with pathogen attachment to intestinal epithelial cells
Immune system modulation	✓ Strengthening of non-specific defense against infection and tumors ✓ Adjuvant effect in antigen-specific immune responses ✓ Enhancement of secretory IgA production
Allergy	✓ Prevention of antigen translocation into blood stream ✓ Prevent excessive immunologic responses to increased amount of antigen stimulation of the gut
Blood lipids, heart disease	✓ Assimilation of cholesterol within bacterial cell ✓ Increased excretion of bile salts due to deconjugation by bile salt hydrolase ✓ Antioxidative effect ✓ Inhibition of Hydroxyméthylglutaryl-CoA reductase (key enzyme of the cholesterol synthesis)
Antihypertensive effect	✓ Bacterial peptidase action on milk protein results in antihypertensive tripeptides ✓ Cell wall components act as ACE inhibitors
Urogenital infections	✓ Adhesion to urinary and vaginal tract cells ✓ Colonization resistance ✓ Inhibitor production (H_2O_2, biosurfactants)
Anti-colon cancer effect	✓ Antimutagenic activity ✓ Detoxification of carcinogenic metabolites ✓ Alteration in pro-cancerous enzymatic activity of colonic microorganisms ✓ Stimulation of immune function ✓ Influence on bile salt concentration

PREBIOTICS

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in colon that can improve the host health [47]**.** Prebiotics cannot be digested by pathogenic bacteria [48, 49]. They stimulate or help the growth of probiotic bacteria (lactobacilli and bifidobacteria) and allow them to grow predominantly [50, 51, 52]. Prebiotics are usually polysaccharides or oligosaccharides. They are naturally found in fruits and vegetables as bananas, garlic, wheat, tomatoes, onions, and legumes [53]. The commonly known prebiotics are: inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), soya-oligosaccharides, xylo-oligosaccharides, pyrodextrins, isomalto-oligosaccharides, and lactulose [11, 54] (Table 4)**.**

Table 4: Composition of some common prebiotics

Name	Composition
Inulin	Beta (2-1) fructans
Fructo-oligosaccharides	Beta (2-1) fructans
Galacto- oligosaccharides	Oligo-galactose (85%) with some glucose and lactose
Xylo- oligosaccharides	Beta (1-4)-linked xylose
Isomalto- oligosaccharides	Alpha(1-4) glucose and branched alpha(1-6) glucose

Honey as a prebiotic

The fructo-oligosaccharides such as inulobiose, kestose, and nystose have been identified in Malaysian honey samples [55]. The oligosaccharides isomaltose and melezitose have been found in New Zealand honey [56]. The presence of raffinose has been observed in Italian honey [57]. Honey contains many oligosaccharides and low molecular weight polysaccharides that can resist degradation by host enzymes, and thus remain available as nutrient source for the intestinal micro flora (Table 5) thereby acknowledged as a prebiotic material. To classify a food ingredient as a prebiotic, it has to fulfill following conditions: (a) it is not broken down in the stomach or absorbed in the GI tract, (b) it is fermented by the gastrointestinal microflora; and (c) it most importantly, selectively stimulates the growth and/or activity of intestinal bacteria associated with health and well-being [58].

Table 5: Prebiotic components in honey

Prebiotic components	References
Inulobiose Kestose Nystose	Jan Mei *et al*., 2010 [55]
Isomaltose Melezitose	Weston and Brocklebank, 1999 [56]
Raffinose	Oddo *et al*., 1995 [57]

Mechanism of action of a prebiotic

When prebiotics enter the lower part of the gastrointestinal tract, they stimulate the growth and/or activity of health-promoting bacteria in the colon. It has been demonstrated that these bacteria generally including lactobacilli and bifidobacteria species protect the host by competing with bacterial and fungal pathogens for availability of nutrients and space and also modulating the immune system. Moreover, during the fermentation of the prebiotics, researchers showed that short chain fatty acid (SCFA) such as acetic, propionic and butyric acids are released and can serve as an energy source for the mucosal cells [59].

SYNBIOTIC

The concept synbiotic is used when both probiotics and prebiotics are contained in a product. In this product, prebiotic compounds selectively favor the growth and activity of the probiotic bacteria [60].

Synbiotic products using honey as prebiotic

Honey has a potential prebiotic activity due to its oligosaccharides [61, 62]. The stimulation of probiotic growth by honey has been reported by many research groups.

Pandiyan showed that honey maintains the level of *L. acidophilus* above the therapeutic level (10^6 CFU/ml) during 15 days of storage in ice cream [63]. Another study demonstrated the stimulative effect of 5% and 7% of honey on the growth of Bifidobacteria and Lactobacilli [64, 65]. Shamala also noted that *Lactobacillus acidophilus* and *L. plantarum* had higher viable counts in a medium containing diluted honey (equivalent to 1% sugar concentration; floral source unknown) than in a medium with sucrose (1%) or a

mixture of glucose (0.5%) and lactose (0.5%). An *in vivo* study conducted in the same study, also showed that viable counts of lactic acid bacteria from both small and large intestines of rats fed with honey were markedly higher than those from rats fed with sucrose [66]. In the same way, Ezz El-Arab [67] demonstrated that the colon bifidobacteria and lactobacilli counts in male swiss albino mice were markedly increased in the group receiving food supplemented with a monofloral (cotton) honey. Riazi and Ziar observed the protective effect of honey on the cell viability of lactic acid bacteria during 28 days of refrigerated storage [68]. Addition of honey in skim milk and soymilk supports the growth of *B. longum* BB536, *B. longum* Bb-46 and *B. lactis* Bb-12 [56, 69]. Ustunol in the research project funded by the National Honey Board and conducted at Michigan State University found that honey enhanced the growth, activity, and viability of commercial strains of bifidobacteria typically used in the manufacture of fermented dairy products. However, this effect was strain-specific. The author reported a synergistic effect among the carbohydrate components of honey in promoting growth and activity of bifidobacteria. He also observed that the effect of honey on the growth and activity of intestinal *Bifidobacterium* spp. was similar to that of commercial oligosaccharides (FOS, GOS, and inulin) [70]. Sanz showed that honey-derived oligosaccharides had similar Prebiotic Index (PI) values with commercial oligosaccharide FOS [61].

Honey, consumed by humans since ancient times, is one of the few products which have conserved its natural character. It is this quality that is searched by most of the health conscious consumers. Honey had been used in almost all civilizations for centuries as a traditional medicine. Moreover, its production does not require special equipment since it is a natural food produced by honeybees and therefore man is involved mostly for harvesting. Although the high osmotic pressure and low pH of honey may not be favorable for the growth and viability of probiotic lactic acid bacteria, it is seen that honey has good prebiotic properties which enhance the viability of probiotics. Hence, honey could be a food of choice for the development of non-dairy probiotic products and thus effectively convey the probiotic lactic acid bacteria to the large intestine where they exert their health benefits. Researchers are working on the production of honey based probiotic products. Hence, Nath showed that probiotic honey beverage made with 25% honey + 10% aloe vera pulp + 25% soy milk + 40% water and fermented by *L. acidophilus* for 6 hours gives viable count of more than 10^9 CFU/ml and has a shelf life of fifteen days under refrigerated condition [71]. On the other hand, Zambou observed that the strain *L. plantarum* 2S remains viable during 28 days of storage in honey (100%) at 4°C [72].

CONCLUSION

Honey is a stable natural food having many beneficial effects on health and has prebiotic oligosaccharides that enhance the viability of probiotic lactic acid bacteria. Hence, it could be used as an exceptional food matrix for making honey based synbiotic formulations.

ACKNOWLEDGEMENT

Authors from University of Dschang, Cameroon acknowledge all members of the Research Team of Microbiology of Lactic Acid Bacteria, Laboratory of Biochemistry, Food Science and Nutrition, University of Dschang, Cameroon for their support. Authors from NIT Rourkela, India are indebted to NIT Rourkela, Odisha, India, Department of Science and Technology (SERB/F/5150/2012-13), and Department of Biotechnology (BT/PR6486/GBD/27/433/2012), Ministry of Science and Technology, Government of India.

REFERENCES

[1] Heenan CN, Adams C, Hoskena RW, Fleet H. LWT-Food Sci Technol 2004; 37: 461-466.
[2] Granato D, Branco GF, Nazzaro F, Cruz AG, Faria JAF. Compre Rev Food Science Food Safety 2010; 9:292-302.
[3] Stanton C, Gardiner G, Meehan H, Collins K, Fitzgerald G, Lynch PB, Ross RP. Am J Clin Nutr 2001; 73, 476S-483S.
[4] Bogdanov S. Bee Prod Sci 2009; 13-17.
[5] Shin HS, Ustunol Z. Food Res Int 2005; 38: 721–728.
[6] Codex Revised codex standard for honey. CODEX STAN 12-1981, 2001; Rev.1 (1987), Rev.2 (2001).
[7] Bogdanov S, Haldimann M, Luginbühl W, Gallmann P. J Apic Res Bee World. 2007; 46 (4): 269-275.
[8] Feller-Demalsy MJ, Vincent B, Beaulieu F. Apidologie 1989; 20(1): 77-91.

[9] Doner LW. Honey. *In* Caballero, B., Trugo, L. and Finglas, P.M. (Eds). Encyclopedia of Food Sciences and Nutrition. Vol. 5. Oxford: Academic Press, 2000. pp. 3125-3130
[10] Kwakman PHS, Te Velde AA, De Boer L. Fed Am Soc Exp Biol J 2010; 7 (24): 2576-2581.
[11] Conway L, Stern R, Tran L. Rural Industries Research and Development Corporation. 2010; Publication No. 09/179 Project No. PRJ-000041, pp. 30.
[12] Bogdanov S, Martin P, Lüllmann C. Apidologie 1997; 29(6): 513-524.
[13] International Honey Commission Harmonised methods of the International Honey Commission. http://www.ihc-platform.net/ihcmethods2009. 2009; Accessed 23-04-2016.
[14] Cordella CB, Militão JS, Clément MC, Cabrol-Bass D. J Agric Food Chem 2003; 51(11): 3234-42.
[15] Marceau J. Noreau J. Houle E. Les HMF et la qualité du miel. Volume 15, numéros 2. Rapport de la Fédération des Apiculteurs du Québec, service de zootechnie, MAPAQ. 1994, pp. 4.
[16] Metchnikoff E. Lactic acid as inhibiting intestinal putrefaction. *In*: The prolongation of life: Optimistic studies. *W. Heinemann, London*. 1907; pp. 161-183.
[17] FAO/WHO Guidelines for evaluation of probiotics in food. Report of a Joint Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. *London, Ontario, Canada.* 2002; April 30 and May 1.
[18] Sanders ME, Huis in't Veld. Anton Leeuw Int J G 2000; 76: 293-315.
[19] Ouwehand AC, Saliminen S, Isolauri E. Anton Leeuw Int J G 2002; 82: 279-289.
[20] Heyman M, Menard S. Cell Mol Live Sci 2002; 59: 1151-1165.
[21] Ahmadi F. Glob Vet 2011; 5: 471-475.
[22] Zambou NF, Sieladie DV, Fonteh AF, Kaktcham PM, El Soda M. Curr Res J Biol Sci 2012; 4: 143-152.
[23] Caplice E, Fitzgerald GF. Int J Food Microbiol 1999; 50: 131–149.
[24] Sieladie DV, Zambou NF, Kaktcham PM, Cresci A, Fonteh F. Innov Romanian Food Biotechnol 2011; 9: 12-28.
[25] Pedone CA, Bernabeu AO, Postaire ER, Bouley CF, Reinert P, Cirde. Int J Clin Pract 1999; 53: 179-184.
[26] Weizman Z, Ghaleb A, Alsheikh A. Pediatrics 1999; 115:5-9.
[27] Cani P, Neyrinck AM, Fava F, Knauf K, Burcelin RG, Tuohy KM. Diabetol 2007; 50: 2374-2383.
[28] Cani P, Delzenne NM, Amar J, Burcelin R. Pathol Biol 2008; 56: 305-309.
[29] Sanders ME. Nat Rev 2003; 61:91-99.
[30] Corthesy B, Rex Gaskins H, Mercenier A. Cross-talk between probiotic bacteria and the host immune system. J Nutr 2007; 137: 781S-790S.
[31] Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Lancet 2001; 357: 1076-9.
[32] Isolauri E. Am. J Clin Nutr 2001; 7:444S-450S.
[33] Ong L, Shah NP. LWT Food Sci Technol 2008; 41: 1555-1566.
[34] Rhyänen EL, Pihlanto-Leppälä A, Pahkala E. Int Dairy J 2001; 11: 441-447.
[35] Pawan, Bhatia. J Clin Diag Res 2007; 1: 467-475.
[36] Kumar R, Grover S, Batish VK. Brit J Nutr 2011; 105: 561–573.
[37] James W, Anderson MD, Stanley E, Gilliland. J Am College Nutr 1999; 18 (1): 43–50.
[38] Ratna SM, Prashant C, Kalpana D, Sekhar B, Kaiser J. Biol Med 2009; 1 (4): 4.
[39] Liong MT, Shah NP. J Dairy Sci 2005; 88: 55–66.
[40] Gilliland SE, Speck MJ. Appl Envt Microbiol 1977; 33: 15–18.
[41] Mahrous H, Shaalan UF, Ibrahim AM. Int Res J Microbiol 2011; 7: 242-248.
[42] Suzuki Y, Kaizu H, and Yamauchi Y. Anim Sci Technol 1991; 62: 565-571.
[43] Fukushima M, Nakano M. Brit J Nutr 1996; 76: 857-867.
[44] Gilliland SE, Nelson CR, Maxwell CV. Appl Environ Microbiol, 1985; 49: 377-381.
[45] Sanders ME. Food Tech 1999; 53(11): 67-77.
[46] Nagpal R, Kumar A, Kumar M, Behare PV, Jain, Yadav H. Fed Eur Microbiol Soc (FEMS) Microbiol Lett 2012; 334: 1–15.
[47] Gibson GR, Roberfroid MB. J Nutr 1995; 125: 1401–1412.
[48] Annika M, Tarja S, Outi V. World Patent 2002; no. WO02060276.
[49] Femia AP, Luceri C, Dolara P, Giannini A, Biggerei A, Salvadori M, Clune Y, Collins KJ, Paglierani M, Caderni G. Carcinogenesis 2002; 23: 1953-1960.
[50] Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati J, Pochart P, Marteau P, Flourié B, Bornet F, Rambaub J-C. J Nutr 1999; 129: 113-116.

[51] Flamm G, Glinsmann W, Kritchevsky D, Prosky L, Roberfroid M. Crit Rev Food Sci Nutr 2001; 41, 353-362.
[52] Wang Y, Xu N, Xi A, Ahmed Z, Zhang B, Bai X. Appl Microbiol Biotechnol 2009; 2: 341-347.
[53] Crittenden R, Payne MJ. Nutrition News. Facts and functions of prebiotics, probiotics and synbiotics. pp 1-2. Department of Human Nutrition, K-State Research and Extension, Kansas State University; Prebiotics.*In*: Lee YK, Salminen S, eds. *Handbook of Probiotics and Prebiotics*. 2008; Wiley-Interscience, 2nd ed; 2008: Chapter 4, pp 535-582.
[54] FAO. FAO Technical Meeting on Prebiotics, http://www.aat.taa.eu.pdf accessed 2007; 27-04-2016.
[55] Jan Mei S, Mohd Nordin MS. and Norrakiah AS. Int Food Res J 2010; 17: 557-561.
[56] Weston RJ, Brocklebank LK. Food Chem 1999; 64: 33-37.
[57] Oddo LP, Piazza AG. *Apidologie*. 1995; 26:453-465.
[58] Gibson GR. Am Soc Nutr Sci 1999; 129:1438S-1441S.
[59] Haddadin MSY, Nazar I, Jamal Abu Raddad S, Robinson RK. Pak J Nutr 2007; 6 (6): 693-697.
[60] Schrezenmeir J, De Vrese M. Am J Clin Nutr 2001; 73:361S–4S.
[61] Sanz ML, Polemis N, Morales V, Corzo N, Dvakoularakou A, Gibson GR. J Agric Food Chem 2005; 3: 2914-2921.
[62] Rosendale DI, Maddox IS, Miles MC, Rodier M, Skinner M, Sutherland J. Int J Food Sci Tech 2008; 43(12): 2257–2267.
[63] Pandiyan C, Annal Villi R, Kumaresan G, Murugan B, Gopalakrishnamurthy TR. Int Food Res J 2012; 19(2):441-446.
[64] Kajiwara S, Gandhi H, Ustunol Z. J Food Prot 2002; 65(1), 214–8.
[65] Amiri ZR, Khandelwal P, Aruna BR. Adv Biosci Biotechnol 2010; 1:224-231.
[66] Shamala TR, Shri JY, Saibaba P. Lett Appl Microbiol 2000, 30: 453-455.
[67] Ezz El-Arab, Girgis AM, Hegazy, Abd El-Khalek AB. BMC Comple Alt Med 2006; 6:6-12.
[68] Riazi A, Ziar H. Afr J Microbiol Res 2012; 3: 486-498.
[69] Slačanac V, Lučan M, Hardi J, Krstanović V, Komlenić. Czech J Food Sci 2012; 30(4):321–329.
[70] Ustunol Z. http://www.honey.com/uploads/general.pdf, 2007; accessed 27-04-2016.
[71] Nath AH, Ukkuru MP, Kumari MKS. Int J Appl Pure Sci Agric 2015, 9: 1-9.
[72] Zambou NF, Moumbe FGP, Kaktcham PM. J Microbiol Biotechnol Food Sci 2013; 3: 226-231.