

Short communication

# Effect of acacia (*Robinia pseudo-acacia* L.) honey on the characteristic microflora of yogurt during refrigerated storage

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## Abstract

The primary purpose of this research was to monitor the influence of acacia honey addition to yogurt milk on survival of the microbial flora of yogurt during refrigerated storage for 6 wk. Results showed that the presence of honey at 1.0% to 5.0% (w/v) did not significantly influence ( $P > 0.05$ ) the viability of characteristic microorganisms (i.e., *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) in yogurt during storage at 4 °C. Similarly, honey had no effect on pH and lactic acid levels of the final products. Despite these findings, enrichment of yogurt with honey is recommended because honey is a natural sweetener that possesses a wide range of beneficial nutritional properties. In addition, at a rate of approximately 3.0% (w/v), it highly improves the sensory quality of the finished product without having a detrimental effect on characteristic lactic acid bacteria.

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## 1. Introduction

Yogurt is a well known fermented dairy food, which is usually manufactured from cow's milk with or without the addition of some natural derivatives of milk, and with the gel structure being the result of coagulation of the milk proteins by lactic acid produced by *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) (Robinson, 2003). Yogurt must contain an abundant and viable microflora of starter origin at the time of consumption, and a definition along these lines is enshrined in the food laws of many countries, with minimum values ranging between  $10^6$  and  $10^8$  CFU/g (Gläser, 1992; Anonymous, 2004).

In recent years, there has been increasing interest in the use of natural food additives and incorporation of health-promoting substances into the diet. Honey, which is a natural syrup containing primarily fructose (38.4%) and glucose (30.3%), has been gaining interest as a substitute sweetener in various foods because of its healthy image (Chick et al., 2001; Ustunol and Gandhi, 2001). Its low pH of approximately 3.9 makes honey

compatible with many food products in terms of acidity. In addition, honey has the ability to decrease the sourness of solutions. This function of honey can serve to increase consumer acceptability of acidic products such as yogurt. Nevertheless, honey–yogurt combinations are relatively uncommon (Brown and Kosikowski, 1970; Roumyan et al., 1996). A plausible explanation for this is that honey is known to have inhibitory effects on lactic starter cultures (Čurda and Plocková, 1995; Roumyan et al., 1996). Factors in honey contributing to its antibacterial nature have clearly not been fully understood (Taormina et al., 2001). These may include the high sugar content, which limits the amount of water available to microorganisms for growth, the relatively high acidity, the presence of organic acids, and the presence, at low concentrations, of hydrogen peroxide (Mundo et al., 2004). However, it is worth noting that, depending on floral sources of the honey, its antimicrobial characteristics reportedly vary (Molan, 1992).

Data on the capability of yogurt starter organisms to metabolize honey are relatively sparse in the scientific literature (Chick et al., 2001). Therefore, the aim of this study was to investigate the ability of *S. thermophilus* and *L. bulgaricus* to grow in the presence of 1.0% to 5.0% (w/v) acacia (*Robinia pseudo-acacia* L.) honey during yogurt fermentation and

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survive in honey-containing yogurts during 6 wk of refrigerated storage at 4 °C. Acacia honey was selected because of its abundant supply in Hungary and its extremely high commercial value. Acacia honey, known for its delicate, herbaceous flavor and pale yellow color, is made of nectar gathered solely from acacia blossoms.

## 2. Materials and methods

### 2.1. Manufacture and storage of yogurts

A 12% (w/v) nonfat dry milk solution (NDM; 4 L) was prepared and divided into four equal portions. The process milks thus obtained were pasteurized at 90 °C for 10 min, then cooled to 45 °C and fortified with acacia honey (Apimel-R Kft., Tiszafüred, Hungary) at levels of 1.0%, 3.0%, or 5.0% (w/v), whereas the fourth batch was devoid of honey and served as control. Next, the NDM with and without honey was inoculated with YC-350 yogurt culture (Chr. Hansen A/S, Hørsholm, Denmark), kindly supplied in freeze-dried direct vat set form by the Hungarian Dairy Research Institute Inc. (Mosonmagyaróvár, Hungary). Incubation took 3.5 h at 42.5 °C to reach a pH value of 4.5 to 4.6. Thereafter, the yogurts were cooled to 15 °C in ice water and were filled into 4×21 sterile, tightly capped centrifuge tubes (30 ml; Sarstedt Aktiengesellschaft and Co., Nümbrecht, Germany). After 24 h of cooling at 8 °C (d 0), the samples were stored at refrigeration temperature (4 °C). The entire experimental program was repeated twice.

### 2.2. Microbiological analysis

Three tubes of all four products were taken at each sampling time, i.e., after 0, 7, 14, 21, 28, 35, and 42 d of storage. Samples were aseptically removed from centrifuge tubes and diluted by mixing 10 ml with 90 ml of 0.1% peptone water. Further dilutions were made as required. The standard pour plate method was employed to determine the viable cell counts of starter organisms.

M17 agar (Oxoid Ltd., Basingstoke, UK) was used to enumerate *S. thermophilus*. The pH of the medium was 7.1±0.1. The inoculated plates were incubated at 37 °C for 48 h under aerobic conditions. *S. thermophilus* formed lenticular colonies with a diameter of 1 to 2 mm (Anonymous, 1997). Colony-forming units (CFU), expressed as log per milliliter, were used to report survival of streptococci. The streptococci identified on the basis of colonial type were confirmed by microscopic examination using a Zeiss KF 2 binocular microscope (Carl Zeiss AG, Göttingen, Germany). *S. thermophilus* were Gram-positive, spherical or ovoid cells (0.7 to 0.9 µm in diameter) appearing in pairs or in long chains (Anonymous, 1997).

Acidified MRS agar (Oxoid) with pH 5.4 was used for enumeration of *L. bulgaricus*. The plates were incubated at 37 °C for 72 h. Anaerobic culture jars (2.5 L) were employed to generate anaerobic conditions, atmospheric oxygen being absorbed by means of AnaeroGen AN 25 sachets (Oxoid). *L. bulgaricus* formed 1 to 3 mm diameter lenticular, often sharp-shaped colonies (Anonymous, 1997). The counts were expressed as log

CFU/ml. The lactobacilli identified were corroborated by observation under a Zeiss KF 2 light microscope (Carl Zeiss). *L. bulgaricus* were Gram-positive, nonmotile, nonsporeforming, generally short rods, which, however, sometimes appeared in longer forms (Anonymous, 1997).

Because the initial viable cell counts of starter organisms in the four product formulations slightly varied prior to refrigerated storage (immediately after a 24-h cultivation and cooling), percentage viabilities of both *S. thermophilus* and *L. bulgaricus* in the presence of different concentrations of honey were calculated as described by Ustunol and Gandhi (2001): % viability = (CFU at *n* wk of storage/initial CFU)×100.

### 2.3. Measurement of acidity

The pH value of samples was determined at room temperature with an HI 8521 pH-meter and combined glass electrode (Hanna Instruments Deutschland GmbH, Karlsruhe, Germany) standardized with pH 4.01 and 7.01 standard buffer solutions (Merck KGaA, Darmstadt, Germany).

Samples (20 ml) were titrated with 0.1 M NaOH, using phenolphthalein as the indicator. The amount of NaOH used (in milliliter) was multiplied by two, and titratable acidity was thus obtained in Soxhlet-Henkel degrees (SH). Multiplied by 0.0225, the SH values were expressed as percentage of lactic acid.

### 2.4. Statistical analysis

All the data obtained were subjected to ANOVA using the general linear model procedure of STATISTICA data analysis software system, version 6.1 (StatSoft Inc., Tulsa, OK, USA). Significant differences among the log CFU/ml, pH, or lactic acid percentage means were determined by using Duncan's multiple comparison test at  $P<0.05$  (StatSoft).

## 3. Results and discussion

Various additives of nondairy origin are used in the manufacture of milk products because of their beneficial contribution to the sensory, therapeutic, or other properties of dairy foods (Varga and Szigeti, 1998; Varga et al., 2002; Molnár et al., 2005). However, some of these substances may contain spoilage microorganisms, thereby negatively affecting the shelf life of finished products. For instance, yeast spoilage may be a major problem in fermented milks when products are supplemented with honey, which is a source of infection and also provides nutrients for yeast growth and fermentation (Jakobsen and Narvhus, 1996). Because in the present study honey was mixed into the pasteurized yogurt milk right after cooling to just above the desired incubation temperature in order to avoid losing some of honey's heat-labile bioactive substances, its hygienic level was of great importance. Therefore, the microbiological quality of acacia honey was determined before use, and it was found to be just excellent, with a mean total plate count not exceeding 10 CFU/g (data not shown). Yeasts, molds, sulfite-reducing clostridia, and *Salmonella* spp. were not detected in the jar of honey used for this trial. These results are well within the

range of current industry experience where the bacterial levels of finished honey tend to vary from 1 to 5000 CFU/g (Snowdon and Cliver, 1996).

Before the start of production and storage trials, preliminary studies were conducted to see the effect of honey addition to yogurt milk on pH during fermentation. The pH of milks dropped as expected over the 6 h of incubation and no significant differences ( $P > 0.05$ ) in pH were observed among the treatments, indicating that honey neither supported nor impeded lactic acid production by *S. thermophilus* and *L. bulgaricus* at this stage (data not shown).

Thereafter, viability of the starter organisms in control and honey yogurts during 42 d of refrigerated storage at 4 °C was monitored at 7-d intervals. The addition of honey to yogurt milks did not significantly influence ( $P > 0.05$ ) the growth and survival of streptococci during production and subsequent refrigerated storage of yogurts (data not shown). *S. thermophilus* was present at sufficiently high levels both at the beginning and at the end of the 6-wk storage period. Food regulations in Hungary require fermented milks to contain viable lactic acid bacteria of starter origin at concentrations of at least  $10^7$  CFU/g at the time of consumption (Anonymous, 2004). Ranging between 8.28 and 8.81 log CFU/ml, even the counts of streptococci largely exceeded this value throughout the entire storage period.

Although likewise exceeding by far the legally required minimum value of  $10^7$  CFU/g (Anonymous, 2004), the initial viable counts of *L. bulgaricus* were found to be approximately 0.4 log cycles lower than those of *S. thermophilus* (data not shown). Similar to what was experienced with streptococci, the addition of honey had no significant effect ( $P > 0.05$ ) on the viability of *L. bulgaricus* either. After 6 wk of storage, viability retention of lactobacilli in the final products ranged between 36.3% and 42.3%. These results are in contrast to those of Roumyan et al. (1996), who found a considerable inhibition in the growth of *L. bulgaricus* when testing the influence of honey addition on the starter organisms in Bulgarian yogurt.

As a result of the aforementioned observations, all four experimental yogurt formulations fulfilled the legal requirements in terms of the levels of viable starter organisms present during the whole storage period, with no significant differences ( $P > 0.05$ ) among the various treatments (data not shown). These results are consistent with Chick et al. (2001), who reported that honey was neither stimulatory nor inhibitory to growth of and acid production by *S. thermophilus* and *L. bulgaricus* when added to nonfat dry milk at a level of 5% (w/w).

Ranging from 4.29 to 4.37, the initial pH values of yogurts did not significantly differ ( $P > 0.05$ ), but a fall in pH of more than 0.3 unit on average was observed in both control and honey-containing yogurts by the end of the 6-wk storage period (data not shown). Similarly, there were no significant differences ( $P > 0.05$ ) among treatments in lactic acid levels during storage (data not shown). These findings are consistent with those of Medina and Jordano (1995), who reported a pH decline of 0.3 to 0.4 unit in the case of probiotic fermented milks during 36 d of storage at 7 °C.

As for sensory properties, the product formulation with the lowest concentration (i.e., 1.0%, w/v) of honey was weak in

flavor. In contrast, the 5.0% (w/v) level was too sweet and was evaluated as too strong in honey flavor. However, the yogurt samples containing 3.0% (w/v) of honey were found to have optimum sweetness (data not shown).

In conclusion, this is the first report on the viability of *S. thermophilus* and *L. bulgaricus* in honey-enriched yogurt during storage. My findings demonstrated that the presence of honey at 1.0% to 5.0% (w/v) did not significantly influence ( $P > 0.05$ ) the survival of characteristic microorganisms in yogurt during a 6-wk storage period at 4 °C. Similarly, honey had no effect on pH and lactic acid levels of the final products. Nevertheless, enrichment of yogurt (i.e., the process milk used for making yogurt) with honey is recommended because honey is a natural sweetener with a wide range of beneficial nutritional properties. In addition, at a concentration of approximately 3.0% (w/v), it highly improves the sensory characteristics of yogurt without having an inhibitory effect on starter bacteria.

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