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Fermentation of Aqueous Plant Seed Extracts by Lactic Acid Bacteria

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The effects of lactic acid bacterial fermentation on chemical and physical changes in aqueous extracts of cowpea (*Vigna unguiculata*), peanut (*Arachis hypogea*), soybean (*Glycine max*), and sorghum (*Sorghum vulgare*) were studied. The bacteria investigated were *Lactobacillus helveticus*, *L. delbrueckii*, *L. casei*, *L. bulgaricus*, *L. acidophilus*, and *Streptococcus thermophilus*. Organisms were inoculated individually into all of the seed extracts; *L. bulgaricus* and *S. thermophilus* were also evaluated together as inocula for fermenting the legume extracts. During fermentation, bacterial population and changes in titratable acidity, pH, viscosity, and color were measured over a 72-h period at 37°C. Maximum bacterial populations, titratable acidity, pH, and viscosity varied depending upon the type of extract and bacterial strain. The maximum population of each organism was influenced by fermentable carbohydrates, which, in turn, influenced acid production and change in pH. Change in viscosity was correlated with the amount of protein and titratable acidity of products. Color was affected by pasteurization treatment and fermentation as well as the source of extract. In the extracts inoculated simultaneously with *L. bulgaricus* and *S. thermophilus*, a synergistic effect resulted in increased bacterial populations, titratable acidity, and viscosity, and decreased pH in all the legume extracts when compared to the extracts fermented with either of these organisms individually. Fermented extracts offer potential as substitutes for cultured dairy products.

In recent years there has been increased interest in using low-cost, relatively high-quality plant proteins as supplements to or replacements for animal proteins in the diet (13). Fermentation of plant materials offers a promising method of preservation (11). Work has been done to determine the effects of lactic acid bacterial fermentation of aqueous extracts of soybeans (soymilk), and two reviews on this subject exist (16, 18). Less work has been done on other legumes such as peanuts or cowpeas (3, 7, 8). While it has been possible to develop acceptable food products (19, 23), there have been no reports to date comparing similarities and differences in these legume milks and their suitabilities to serve as substrates for fermentation by lactic acid bacteria. The study reported here describes the results of a study designed to make this comparison.

MATERIALS AND METHODS

Seed types and sources. California black-eyed peas (*Vigna unguiculata*) were obtained from the California Bean and Grain Cooperative, Pixley, Calif. Peanuts (*Arachis hypogea* cv. Florunner) and soybeans (*Glycine max* cv. Wright) were obtained from the Georgia Seed Development Commission, Athens, Ga. Sorghum (*Sorghum vulgare*) was obtained from L. W. Rooney, Soil and Crop Science Department, Texas A & M University, College Station, Tex. Upon receipt, the seeds were stored at 3°C and 60% relative humidity until used.

Extraction procedure. A 1-kg quantity of each seed type was submerged in 2 liters of tap water containing 0.5% (wt/vol) sodium bicarbonate and soaked for 18 h at 21°C. The water was then drained, and the seeds were washed with fresh tap water. The washed seeds were coarsely ground, mixed with 5 liters of tap water and held for 5 h at 21°C. After steeping, the extracts were filtered through four layers of cheesecloth and cooled to 5°C. The resulting aqueous solutions are referred to as seed or legume extracts throughout this report.

Bacterial strains and culture methods. Five species of *Lactobacillus* were obtained from the U.S. Department of Agriculture Northern Regional Research Laboratory, Peoria, Ill.: *Lactobacillus helveticus* B-176, *L. delbrueckii* B-455, *L. casei* B-1445, *L. bulgaricus* B-1909, and *L. acidophilus* B-1910. In addition, one strain of *Streptococcus thermophilus* (RR) was supplied by Joseph Frank, Department of Animal Science, University of Georgia, Athens. The bacteria were cultured and maintained in a medium consisting of a 50:50 (vol/vol) mixture of APT broth and cowpea, peanut, soybean, or sorghum extract. Cultures were incubated at 37°C for 48 h before cooling to 5°C for storage between transfers.

Fermentation procedure. The seed extracts were pasteurized in 100-ml quantities for 10 min at 121°C and cooled to 37°C before inoculation. Inocula (1%) consisting of 24-h cultures grown in seed extract-APT medium were used to initiate the fermentation. Each of the six bacterial cultures was added to each of the four seed extracts. Two strains (*L. bulgaricus* and *S. thermophilus*) were also inoculated together (1:1 ratio) into the extracts. All inoculated extracts were incubated at 37°C.

Analysis of seed extracts. The extracts were analyzed for protein content by the Kjeldahl method (1) for total soluble solids by using a microwave moisture balance (CEM Corp., Indian Springs, N.C.) and for total lipids by using a procedure reported by Beuchat and Worthington (4).

Analysis of fermented products. Inoculated extracts were analyzed at various time intervals (0, 8, 24, 32, 48, and 72 h) for microbial population, titratable acidity (expressed as lactic acid), pH, viscosity, and color.

Microbial populations of the extracts inoculated with only one strain were determined by diluting thoroughly mixed samples in 0.1 M potassium phosphate buffer (pH 7.0) followed by surface plating on APT agar. Populations in

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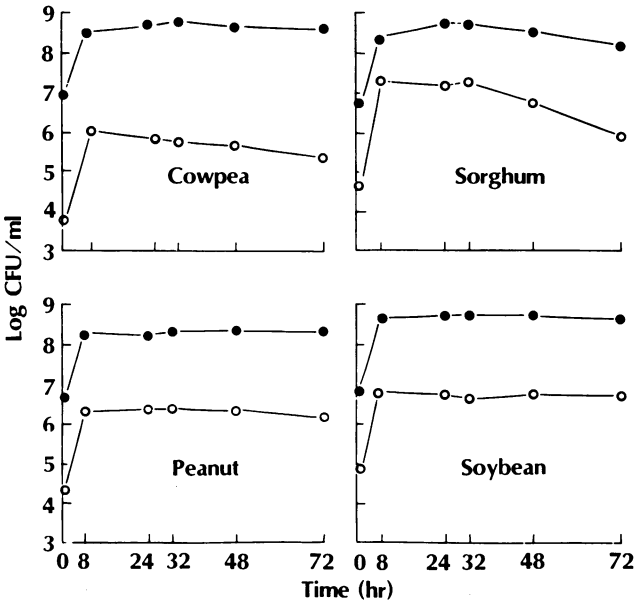

FIG. 1. Log of CFU per milliliter of seed extracts during fermentation with *L. helveticus* (●) and *S. thermophilus* (○).

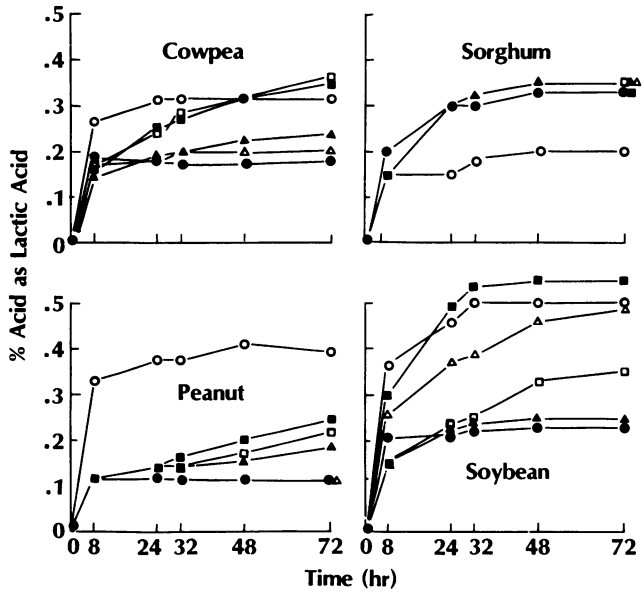

FIG. 2. Percent titratable acidity calculated as lactic acid in seed extracts during fermentation with *L. helveticus* (●), *L. delbrueckii* (■), *L. casei* (□), *L. bulgaricus* (▲), *L. acidophilus* (△), and *S. thermophilus* (○).

extracts inoculated with both *L. bulgaricus* and *S. thermophilus* were also diluted in phosphate buffer but were plated on Lee agar (21) to permit differentiation of lactobacilli and streptococci based on colony color and morphology. Both types of enumeration media were incubated at 37°C under a gaseous atmosphere containing 15% CO_2 for 48 h before colonies were counted.

The titratable acidity was measured by titration of fermented extracts with 0.1 N NaOH to a pH of 8.1; 1% (wt/vol) phenolpthalien in 95% ethanol was used as an indicator. The pH of the fermented samples was also measured before titration.

The viscosity of the samples was measured by using the Brookfield synchrolectric viscometer (model RVT; Brookfield Engineering Laboratories Inc., Stoughton, Mass.) equipped with a TA spindle and helipath stand (model C).

The color of the extracts and fermented products was measured by using a Gardner colorimeter (model XL 800; Pacific Scientific, Gardner Laboratory Division, Bethesda, Md.) with an XL 845 circumferential sensor.

RESULTS AND DISCUSSION

The growth patterns of test organisms shown in Fig. 1 were similar to those reported by other investigators using soybean extracts (17) and are within the ranges reported for fermented cow milk yogurt (9). Growth patterns for *L. delbrueckii*, *L. casei*, *L. bulgaricus*, and *L. acidophilus* were similar to those of *L. helveticus* for each seed extract. Only data for *L. helveticus* and *S. thermophilus* are presented in Fig. 1. Differences in plate counts can be attributed in part to different methods of extract preparation. As Patel et al. (18) have noted, heating times and temperatures to which soybean extracts are subjected can have either inhibitory or stimulatory effects on the growth of lactic acid bacteria.

Titratable acidity. The amount of acid produced by each test organism differed, depending upon the type of seed extract fermented (Fig. 2). A major difference among the species evaluated in this study is in their ability to ferment sucrose (6). Sucrose is present in appreciable quantities in the dried legume seeds (10, 22) and would be extracted during the steeping process. Two of the strains (*L. bulgaricus* and *L. helveticus*) tested cannot ferment sucrose and, indeed, produced less acid than did other species in peanut and soybean extracts. Acid production by these organisms as well as *L. acidophilus* was also retarded in cowpea extract. All lactobacilli produced similar levels of acid in sorghum extract.

Aqueous extract of soybean supported elevated acid production by *L. acidophilus* compared with the other extracts. It is interesting to note that *L. acidophilus* was the only species tested which can ferment raffinose (6), a sugar present at a higher concentration in soybeans than in peanuts or cowpeas. Other differences in acid production can be attributed to the ability of various species to ferment stachyose, which is present in all three legumes evaluated (22, 24). Another factor contributing to different levels of acidity may be the amount of total carbohydrate present in various extracts (Table 1). The amount of total carbohydrate varied among seed types. Different levels of carbohydrate would directly affect theoretical maximum amounts of acid which could be formed by various test organisms.

It has been suggested that acid production in seed extracts may be inhibited by relative levels of saturated and unsatur-

TABLE 1. Composition of aqueous seed extracts

Seed type	Total soluble solids (%)	Protein (%)	Total lipid (%)	Carbohydrate (%)[a]
Cowpea	3.0	1.30	<0.1	1.6
Peanut	3.5	0.95	1.60	0.95
Soybean	3.5	1.10	0.60	1.8
Sorghum	2.8	0.25	ND[b]	ND

[a] Carbohydrate by difference.
[b] ND, Not done.

ated free fatty acids (7), and it is known that such compounds can inhibit growth of lactic acid bacteria (7, 15). A similar phenomenon may have occurred in the present study.

pH. In general, the results of the pH measurements followed an opposite trend to that observed for titratable acidity measurements; i.e., as the acidity increased, the pH decreased (Fig. 3). For example, the amount of acid produced by *S. thermophilus* in the peanut extract exceeded acid production by the other species (Fig. 2). Simultaneously, *S. thermophilus* caused the greatest drop in pH in fermented peanut extract. Those organisms which produced less acid in legume extracts generally caused less decrease in pH.

The relationship between pH and titratable acidity is not always consistent. For example, the amount of acid produced by *L. casei* and *L. delbrueckii* was roughly equivalent in sorghum and cowpea extracts (Fig. 2). On the other hand, the final pH achieved in the sorghum extract was almost one pH unit lower than the final pH in the cowpea extract. One explanation for the difference in final pH can be drawn from the data in Table 1. The concentration of protein was substantially lower in the sorghum extract (0.25%) compared with that in the cowpea extract (1.30%). Since the cowpea extract had about five times the amount of protein that was in the sorghum extract, its capacity to buffer pH change was greater. This would result in less decrease in pH even though the amounts of acid present in the two extracts were similar.

Viscosity. Figure 4 shows the viscosities of extracts fermented for various periods of time. It should be noted that the viscosity of sorghum extract did not increase appreciably with time, and no curd formation was observed. It was decided that the task of developing an acceptable product from sorghum was beyond the scope of this study; thus, the evaluation of sorghum extract as a fermentable substrate was discontinued.

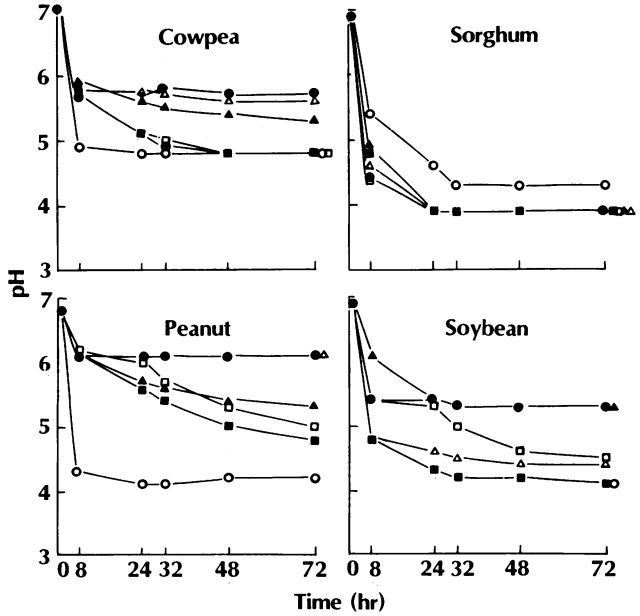

FIG. 3. Change in pH of four seed extracts during fermentation with *L. helveticus* (●), *L. delbrueckii* (■), *L. casei* (□), *L. bulgaricus* (▲), *L. acidophilus* (△), and *Streptococcus thermophilus* (○).

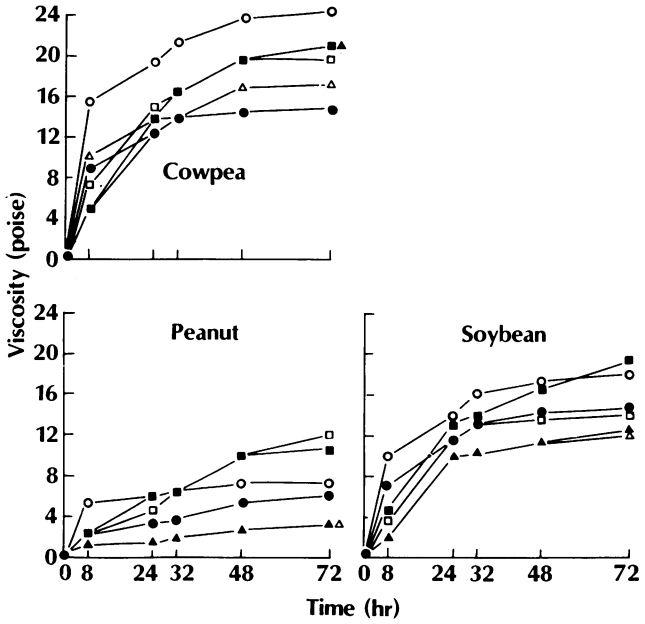

FIG. 4. Viscosity of three seed extracts during fermentation with *L. helveticus* (●), *L. delbrueckii* (■), *L. casei* (□), *L. bulgaricus* (▲), *L. acidophilus* (△), and *S. thermophilus* (○).

Comparison of titratable acidity (Fig. 2) with viscosity (Fig. 4) of fermented extracts reveals that higher acidity was general correlated with increased viscosity. However, this relationship was not as direct as the relationship between titratable acidity and pH. Other factors may play a role in development of increased viscosity. The levels of protein were different in various extracts. Cowpea extract, which contained the highest concentration of protein in any of the seed extracts, was also characterized by the highest viscosity after 72 h of fermentation. Peanut extract, with the least amount of protein, exhibited the smallest increase in viscosity as a result of fermentation. The effect of protein and acid on viscosity can be explained by making an analogy with a well-studied phenomenon, the coagulation of cow milk by the addition of acid. The formation of protein curd in the presence of acid is a well-known occurrence (14). As H^+ ions are produced, they act to neutralize the net negative charges on protein molecules, moving them closer to their isoelectric points. As the charge on the protein molecules decreases, the molecules come into close association and precipitate out of solution, forming a curd. The amount of curd formation is therefore related to the amount and type of protein present as well as the acidity, with more protein and more acid producing a firmer curd.

Color. The change in color (lightness) of the seed extracts as a result of pasteurization and fermentation with *L. bulgaricus* is shown in Fig. 5. The unpasteurized extracts had higher L values than the corresponding pasteurized unfermented extracts; i.e., they were lighter in appearance or more white. Pasteurization caused a decrease in L values, indicating a darkening in color. The most likely explanation for this color change is the formation of Maillard browning reaction products (12). Reasons why the extracts lighten in color upon fermentation are not clear. The phenomenon may be pH related; however, other components in the extract may well play a role. The color change seen here may be similar in nature to one documented by Blovin et al. (5), who

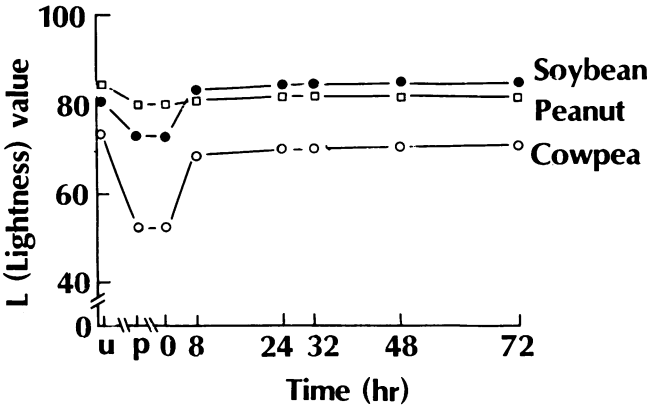

FIG. 5. Relative L values of the three seed extracts; unpasteurized (u), pasteurized (p), and during fermentation with *L. bulgaricus*. Symbols: ●, soybean extract; □, peanut extract; ○, cowpea extract.

observed that when the pH of an aqueous cottonseed paste was adjusted up to pH 10 the paste became darker in color. Perhaps a similar reaction occurs in reverse to lighten the legume extracts when the acid produced during fermentation lowers the pH.

Microbial interactions. It is well documented that a mutually stimulatory effect occurs when *L. bulgaricus* and *S. thermophilus* are grown together in the fermentation of cow milk yogurt (20). *S. thermophilus* stimulates the growth of *L. bulgaricus* by removing oxygen and by producing acid (9). In turn, *L. bulgaricus* stimulates the growth of *S. thermophilus* by promoting the release of free amino acids into the milk (2). When the two species are grown together in the aqueous extract of legume seeds, a similar synergistic effect apparently occurs. The simultaneous culture of these species resulted in a significant increase in the population of *S. thermophilus* but not *L. bulgaricus* in all three legume extracts (Fig. 6). Inhibition by free fatty acids might be masking any benefits *S. thermophilus* might afford to *L. bulgaricus*. Also, *S. thermophilus* might not be able to

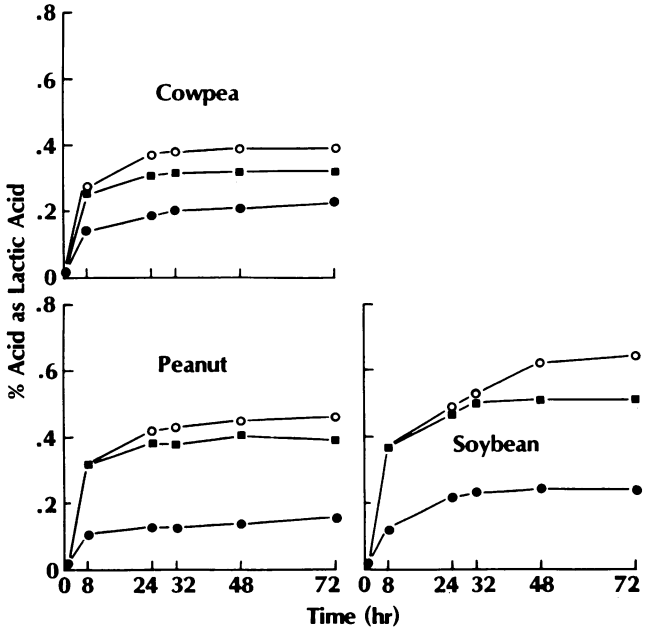

FIG. 7. Percent titratable acidity calculated as lactic acid during fermentation of three legume extracts with *L. bulgaricus* (●), *S. thermophilus* (■), and *L. bulgaricus* and *S. thermophilus* together (○).

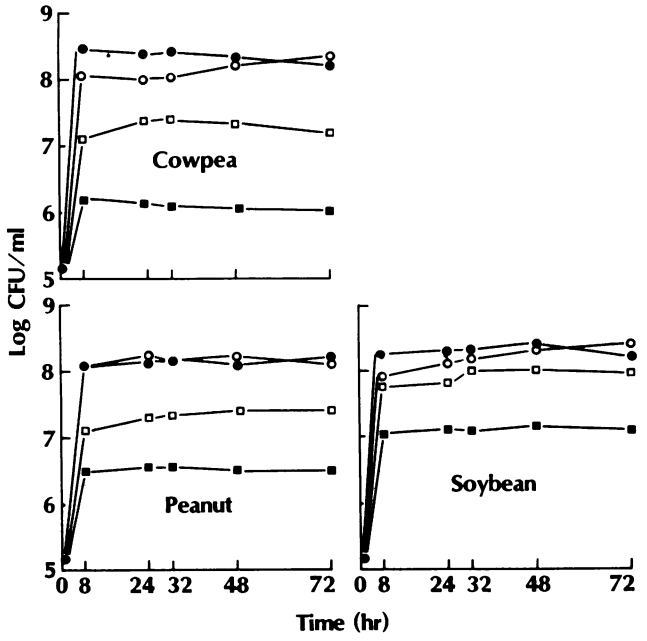

FIG. 6. Change in number of CFU per milliliter of legume extract during fermentation with *S. thermophilus* grown alone (■), *L. bulgaricus* grown alone (●), *S. thermophilus* grown with *L. bulgaricus* (□), and *L. bulgaricus* grown with *S. thermophilus* (○).

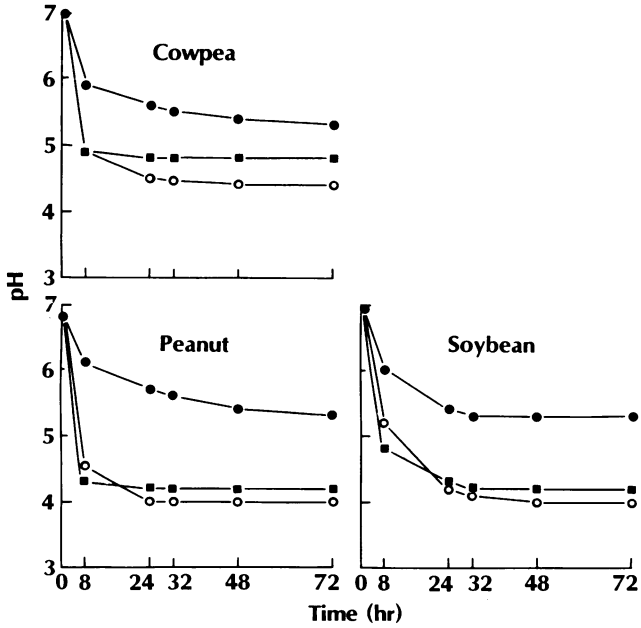

FIG. 8. Change in pH of three legume extracts during fermentation with *L. bulgaricus* (●), *S. thermophilus* (■), and *L. bulgaricus* and *S. thermophilus* together (○).

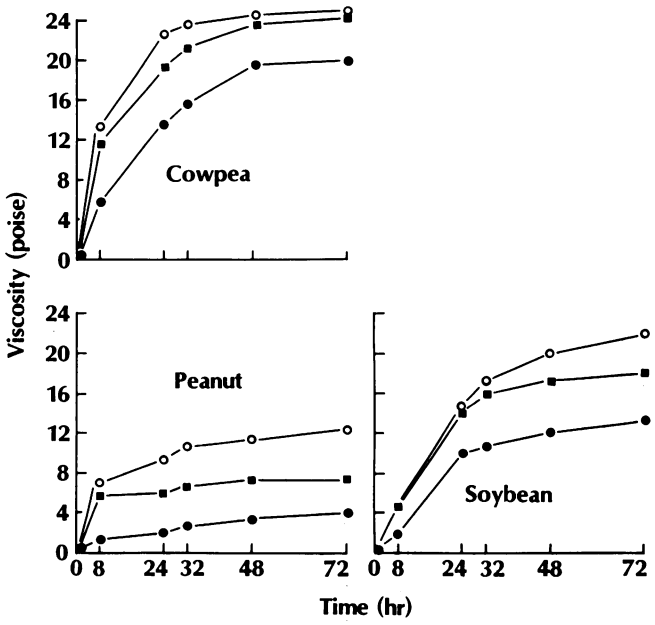

FIG. 9. Increase in viscosity of three legume extracts during fermentation with *L. bulgaricus* (●), *S. thermophilus* (■), and *L. bulgaricus* and *S. thermophilus* together (○).

produce enough acid to cause noticeable stimulation of *L. bulgaricus* growth.

Simultaneous culture of *S. thermophilus* and *L. bulgaricus* also resulted in higher acidities and lower pH values in all the extracts (Fig. 7 and 8, respectively). In addition, the viscosity of extracts inoculated with both species was higher than that of extracts fermented with either *S. thermophilus* and *L. bulgaricus* alone (Fig. 9). This is probably due to an increased acidity leading to more protein coagulation.

In summary, it was observed that fermented seed extracts exhibited different properties, depending upon the seed type and the bacterial species used in fermentation. These properties were influenced by the type of fermentable sugars present in the extract as well as the ability of bacterial species to metabolize them. The amount and type of protein present in extracts affected the buffering capacity and, hence, the resistance to change in pH. The viscosity of the extracts is apparently related to the amount of protein present as well as the amount of acid produced. The extracts became darker upon pasteurization, most likely owing to the production of Maillard browning reaction products, but then lightened again during fermentation. Finally, the synergistic effects of *L. bulgaricus* and *S. thermophilus* that have been noted in the fermentation of cow milk were demonstrated in the fermentation of the seed extracts. It is concluded that peanut and cowpea seeds, in addition to soybeans, offer great potential as sources for the production of fermented yoghurt-like food products.

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