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Inhibitory Effect of Autoclaving Whey-Based Medium on Propionic Acid Production by *Propionibacterium shermanii*

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Propionic acid production by *Propionibacterium shermanii* was compared in pasteurized and autoclaved whey-based media. Propionic acid production decreased with increasing whey concentration in autoclaved media but not in pasteurized media. Increasing the yeast extract concentration from 5 to 10 g/liter greatly reduced the inhibitory effect of autoclaving.

Cheese whey has been used as a substrate in a variety of fermentation processes (7). Most of these processes do not require sterilization of the medium due to rapid fermentation, low pH, or high temperatures or combinations of these factors. Those fermentations that do require sterile medium often use complicated or time-consuming sterilization methods, such as repeated pasteurization (tyndalization) (8) or pasteurization with hydrogen peroxide followed by catalase treatment (2).

Propionibacteria have been used for production of propionic acid (10), vitamin B_{12} (3, 4; M. Lutskova, Int. Dairy Congr., p. 75–77, 1966), and biomass (8) in whey-based media. *Propionibacterium shermanii* grows slowly compared with many other bacteria and prefers neutral pH and mesophilic temperatures. Fermentations with *P. shermanii* are therefore susceptible to contamination problems. This is especially critical if the crude broth is dried and used as a food supplement. The purpose of this investigation was to determine the ability of whey-based media to support growth of *P. shermanii* when sterilized by autoclave.

P. shermanii PS-1 (Chris Hansen's Laboratory, Inc., Milwaukee, Wis.) was maintained in sodium lactate agar stabs (Trypticase peptone [BBL Microbiology Systems, Cockeysville, Md.], 10 g; yeast extract [Difco Laboratories, Detroit, Mich.], 10 g; sodium lactate, 10 g; agar, 20 g; water, 1,000 ml). Inoculated stabs were incubated at 30°C for 96 h and stored at 4°C for up to 6 months. Stab cultures were revived by overlaying with 5 ml of Hansen glucose broth (Trypticase peptone, 20 g; yeast extract, 5 g; glucose, 5 g; water 1,000 ml) and incubating at 30°C for 48 h. Inoculum for experiments was prepared by inoculating 25 ml of Hansen glucose broth in a 50-ml screw-cap flask with 1.0 ml of broth from a revived stab (4%, vol/vol) and incubating at 30°C for 48 h on a rotary shaker at 120 rpm.

The experimental media consisted of 20 to 70 g of dried sweet whey per liter (Teklac; Foremost Food Co., San Francisco, Calif.) and either 5 or 10 g of KAT yeast extract (Stauffer Chemical Co., Westport, Conn.) per liter. The media were adjusted to pH 7.0 with NaOH and dispensed into 50-ml screw-cap flasks (25 ml per flask) containing 0.5 g of $CaCO_3$ as a buffer. Note that the $CaCO_3$ prevented the pH from falling below 5.5 to 5.6, a pH at which propionic acid production continues. The flasks were either autoclaved for 15 min at 121°C and 15 lb/in^2 or pasteurized in a water bath at 65°C for 50 min. The flasks were inoculated with *P. shermanii* grown in Hansen glucose broth (4%, vol/vol) and incubated at 30°C for 72 h on a rotary shaker at 120 rpm. After incubation the broths were assayed for propionic acid on a Packard model 427 gas chromatograph, using a 6-ft (ca. 183-cm) glass column with a 2-mm internal diameter packed with 10% AT-1000 on Chromosorb WAW, 80/100 mesh (Alltech Associates, Arlington Heights, Ill.). Column temperature was 140°C with N_2 carrier gas at a flow rate of 20 ml/min. Injector and flame ionization detectors were at 250°C. Broths were diluted 1:9 with 1% H_3PO_4; the volume injected was 3 μl. An external standard curve was used for quantitation.

In pasteurized media propionic acid production increased as the whey concentration increased up to 40 g of whey solids per liter (Table 1). In media containing 5 g of yeast extract per liter the propionic acid production was 15 to 20% less than in media containing 10 g of yeast extract per liter. This indicates possible nutritional limitation at the lower yeast extract concentration. In contrast, propionic acid production in autoclaved media containing 5 g of yeast extract per liter declined with increasing whey concentration. When yeast extract was increased to 10 g/liter the inhibitory effect of autoclaving the whey solids was greatly reduced. There was, however, a decrease in propionic acid production at 70 g of whey solids per liter.

The decline in propionic acid production with increasing concentrations of autoclaved whey can be explained by two hypotheses. First, vital nutrients (vitamins, amino acids, sugars) may be destroyed when the whey is autoclaved. The yeast extract serves to replace destroyed nutrients directly or inhibit reactions leading to nutrient loss or both, the effect being more pronounced at higher yeast extract concentrations. Second, inhibitory compounds are formed when the whey is autoclaved. The higher the whey concentration, the higher the concentration of inhibitory compounds. The yeast extract in this case would reduce the formation of inhibitory compounds or render them less inhibitory, i.e., bind the inhibitor. Clearly, these hypotheses are not mutually exclusive.

There is evidence from the literature to support both of these hypotheses. Malliard-type browning reactions between lactose and free amino groups on proteins have been demonstrated in milk and milk products. These reactions are accelerated at high temperatures and result in destruction of essential amino acids, vitamins, and lactose as well as the production of toxic substances (5). Lactose is also converted to lactulose [4-(*O*-β-D)-galactopyranosyl-D-fructofuranose] upon autoclaving. The percentage of lactose converted to lactulose increases with increasing lactose concentration.

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TABLE 1. Propionic acid production by *P. shermanii* in pasteurized and autoclaved whey-based media

Whey solids (g/liter)	Yeast extract (g/liter)	Propionic acid (g/liter)[a]	
		Pasteurized	Autoclaved
20	5	5.1	5.2
30		6.5	4.8
40		8.1	3.9
50		8.2	0.6
70		9.2	0.4
20	10	5.9	5.9
30		8.1	7.7
40		10.3	9.1
50		10.4	10.3
70		10.6	7.7

[a] Numbers are averages of two flasks each.

Many organisms are unable to ferment lactulose (9). Further, lactulose at high concentrations is inhibitory to bacterial growth (6). It has also been shown that whey proteins are denatured when autoclaved. This may limit their availability to microorganisms. The extent of denaturization can be reduced by the addition of certain compounds, such as hydrogen peroxide or glucose residue polymers (dextrins), to the medium (1).

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