A Research Note

Test for Susceptibility of Fermented Vegetables to Secondary Fermentation

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ABSTRACT

A simple test is described for determining completeness of vegetable fermentations. Brines of fermented vegetables are adjusted to pH 4.5, inoculated with selected fermentative yeasts in culture tubes, overlaid with petrolatum, and incubated. Fermentation is indicated by separation of petrolatum from the brine due to gas production by the yeasts. Nutrient supplementation of fermented vegetable brines required to support gas production by yeasts and growth of lactic acid bacteria was determined. Absence of fermentable carbohydrate was the indicated reason for brines not being susceptible to secondary fermentation.

INTRODUCTION

CERTAIN FRUITS and vegetables are microbiologically stable provided all fermentable sugars are removed, the pH is 3.8 or below, and the products are stored anaerobically (Fleming et al., 1983). Fermentable sugars are converted to acids, alcohols and other end products by lactic acid bacteria and yeasts during primary fermentation. Incomplete fermentation of sugars during bulk storage can result in secondary yeast fermentation of the products during subsequent storage in consumer or institutional packs. Gaseous spoilage and brine turbidity are causes of economic loss in such products. Economic benefits and improved product quality, therefore, are inherent advantages in successfully fermented vegetables.

A suitable test is needed to determine when fermentation of brined vegetables is complete and when final packaging can be safely done. Analytical determination of residual fermentable sugars would indicate the liklihood of secondary fermentation. However, a direct method for determining susceptibility of fermented vegetables to secondary fermentation could provide more reliable information.

A simple, direct method is described for determining the stability of fermented vegetables to secondary fermentation by yeasts. Also, nutrient supplementation of fermented vegetable brines required to support gas production by yeasts and growth of *Lactobacillus plantarum* was determined.

MATERIALS & METHODS

BRINES of fermented vegetables from a companion study (Fleming et al., 1983) were used in this study and included those from fermented cucumbers, green beans, carrots, green and red bell peppers, red beets, and green tomatoes. The brines were taken immediately after fermentation, as indicated by cessation of base addition by the pH controller, which varied among products from 8–35 days (Fleming et al., 1983). Fermentation brines were adjusted to pH 4.5 with NaOH, centrifuged and the supernatant decanted and filter-sterilized through a 0.45μ membrane filter. Aliquots (7 ml) of the sterile broth were dispensed into sterile, disposable, 16 mm diameter culture tubes. Sterility of the filtered broth was verified by incubating control tubes at 30°C for 10 days.

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To determine nutrients essential for gas production by yeasts or growth of *L. plantarum* in the spent vegetable brines, the filter-sterilized broths were supplemented with 1 ml per tube of either sterile vitamin-free yeast base (VFYB, Difco, 10X concentration), yeast carbon base (YCB, Difco, 10X concentration), yeast nitrogen base (YNB, BBL, 10X concentration), glucose (10% solution), lactobacilli MRS broth (Difco, 10X concentration), or sterile water.

One set of duplicate tubes of all media was inoculated with two drops of washed cell suspensions of yeasts and another set of tubes with *L. plantarum* WSO. Yeast cells for inoculation were grown in malt extract broth (BBL) for 48 hr at 24°C on a shaker, washed three times by centrifugation at 7000 rpm for 10 min, and resuspended in sterile 0.85% saline ($O.D._{650\ nm}$ = ca. 1.2). Yeasts included *Saccharomyces rosei* NRRL Y1567, *Hansenula subpelliculosa* NRRL Y1096, two unidentified yeasts from green bean fermentation and one yeast from red beet fermentation. The five yeasts were grown separately and inoculated singly or in a mixture of the five into the filter-sterilized vegetable brines. The *L. plantarum* cells were grown in MRS broth (Difco Laboratories, Detroit, MI) containing 2% NaCl for 16 hr at 30°C, washed similarly, and adjusted to $O.D._{650\ nm}$ of 0.2–0.8.

The broth tubes, containing appropriate supplements and yeast inoculum, were overlaid with ca. 3 ml of sterile white petrolatum (Fisher Scientific Co.) and incubated at 30°C. Gas production from yeast fermentation was evidenced by displacement of the solidified petrolatum plug with complete separation from the fermentation broth.

The other set of tubes was supplemented as above, but was inoculated with washed *L. plantarum* cells. These tubes, which were not overlaid with petrolatum, were incubated at 30°C. After incubation, the cells were washed 2X with 0.85% NaCl, resuspended to the initial volume, and the optical density at 650 nm determined after 10 days. The cells were washed to separate them from the fermentation brines to avoid misrepresentation of the cell density because of deep coloration in certain brines, especially beets.

RESULTS & DISCUSSION

THE pH-ADJUSTED, spent brines of fermented green beans, cucumbers, bell peppers, and green tomatoes were not fermented by any of the yeasts tested (Table 1). None of these brines contained residual fermentable sugars (Fleming et al., 1983). These products were shown to be microbiologically stable over a 1-yr period in a related study (Fleming et al., 1983). The absence of fermentable carbohydrate apparently accounted for the failure of these brines to ferment because supplementation with glucose resulted in gas production. Neither the addition of vitamins nor nitrogen compounds was essential for gas production. The adjusted brines of beets and carrots were fermented by yeasts when no fermentable carbohydrate was added. Lactic fermentation in these brines had stalled or greatly slowed. These brines did not contain detectable amounts of glucose or fructose, but did contain sucrose (beets, 2.8%; carrots, 0.91%; Fleming et al., 1983). The fermented beets and carrots were shown to undergo a secondary gaseous fermentation during storage (Fleming et al., 1983).

Growth of *L. plantarum*, measured turbidimetrically, occurred only in the spent brine that contained residual fermentable sugar (beets, carrots), or when the brines were supplemented with glucose or nutrients containing fermentable carbohydrate (Table 2). Slight growth occurred when the brines were supplemented with VFYB and YCB, both of which contained fermentable carbohydrates. However,

Table 1—Nutrients essential for gas production by yeasts in fermented vegetable brines

Medium supplement[a]	Missing nutrient(s) in supplement	Fermented vegetable						
		Beans	Beets	Carrots	Cucumbers	Peppers, green	Peppers, red	Tomatoes
VFYB	Vitamins	+[b]	+	+	+[b]	+	+	+
YCB	Nitrogen compounds	+	+	+	+	+	+	+
YNB	Ferm. carbohy.	–	+	+	–	–	–	–
GLU	Vitamins, nitrogen compounds	+	+	+	+	+	+	+
MRS	None	+	+	+	+	+	+	+
Water	All	–	+	+	–	–	–	–

[a] VFYB = vitamin-free yeast base; YCB = yeast carbon base; YNB = yeast nitrogen base; GLU = glucose; MRS = deMan, Rogosa, Sharpe broth.
[b] One of the five yeast cultures did not produce gas in this medium.

Table 2—Nutrients essential for growth of L. plantarum in fermented vegetable brines

Medium supplement[a]	Missing nutrient(s) in supplement	L. plantarum growth, $O.D._{650\ nm}$						
		Beans	Beets	Carrots	Cucumbers	Peppers, green	Peppers, red	Tomatoes
VFYB	Vitamins	0.14	0.71	0.21	0.05	0.18	0.15	0.25
YCB	Nitrogen compounds	0.22	0.68	0.27	0.10	0.20	0.20	0.23
YNB	Ferm. carbohyd.	0.05	0.87	0.28	0.00	0.01	0.02	0.06
GLU	Vitamins, nitrogen compounds	0.14	0.60	0.28	0.15	0.20	0.18	0.18
MRS	None	0.72	1.00	1.80	0.62	0.65	0.60	0.60
Water	All	0.05	0.70	0.17	0.00	0.03	0.04	0.02

[a] See footnote a for Table 1 for identity.

growth was much greater when the spent broth was supplemented with MRS nutrients. These findings suggest that *L. plantarum* growth was limited in unsupplemented spent brines by absence of fermentable carbohydrates as well as other nturients, and that inhibitory compounds were not the cause of growth cessation since supplmentation with MRS nutrients resulted in good growth.

We think that a simple test for gas production such as that described herein could be used as a quality control measure to establish the completeness of vegetable fermentations. Such information would be valuable in ascertaining when products can be safely transferred from bulk storage into consumer packages, without fear of subsequent gaseous spoilage.

For quality control purposes, the test for gas production could be simplified from that described herein. Brine centrifugation and sterile filtration could be omitted. Thus, the procedure would involve adjustment of the brine to pH 4.5, inoculation with a test yeast(s), overlayment of the brine in a suitable tube with petrolatum, and incubation for sufficient time to test for gas production. The inclusion of yeast species other than those tested may be necessary for products that will not support growth and gas production by the species mentioned, but will support growth by species adapted to the product. In such cases, it would be advisable to include in the tests yeasts actually responsible for gaseous spoilage in such products. If the brine sample is taken during active fermentation, the concentration of fermentable sugar in the brine may not represent that still remaining in the vegetable. In this case, representative samples of vegetable and brine should be blended, and the test for gas production determined using the blended material.

The method used for detection of gas production is a modification of that described by Gibson and Abdel-Malek (1945) for heterofermentative lactic acid bacteria. They used melted and cooled nutrient agar to overlay inoculated, semisolid media contained in culture tubes. Gas production is detected by a separation of the agar overlay from the semisolid media. We used petroleum jelly to overlay fermented brines because it did not contain growth nutrients, but did provide an adequate seal. The fermented brines were adjusted to pH 4.5 to favor rapid growth and gas production by yeasts. Gibson and Abdel-Malek (1945) indicated that rapid growth of the organism is essential for the test, presumably to avoid a gradual dissipation of gas.

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