

# Cellular Component of *Kluyveromyces fragilis* Related to Survival During Frozen Storage

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## Story in Brief

Concentrated cultures of *Kluyveromyces fragilis* were prepared by resuspending cells of the yeast in 10 percent non-fat milk solids and freezing them at  $-19^{\circ}\text{C}$  for 24 hr. The eight strains of *K. fragilis* considered in this study exhibited significant differences in survival following this storage period ( $P < .005$ ). There was a significant ( $P < .05$ ) relationship between the cellular content of dodecanoic acid and survival at  $-19^{\circ}\text{C}$ . As the percentage of dodecanoic acid in the cells increased, the percentage of survivors after freezing increased.

## Introduction

*Kluyveromyces fragilis* is a yeast culture that can be used to culture cheese whey to reduce the load on waste treatment systems when the whey is discharged from a cheese plant. The whey protein and yeast cells can be recovered for use as a food or feed ingredient. To provide adequate cultures for this process it would be desirable to have frozen concentrated cultures available.

Cells of *K. fragilis* survive freezing at  $-19^{\circ}\text{C}$  better than freezing in liquid nitrogen (Bostian and Gilliland, 1979). However, all strains of the culture did not survive equally well at  $-19^{\circ}\text{C}$ . The process of freeze-thawing yeast cells apparently causes damage to the cell membrane which could easily involve lipid fractions of the membrane.

Little or no information is available indicating the effect of cellular fatty acid composition on the survival of yeast cultures during freezing. However, Smittle *et al.*, 1974, have shown that the fatty acid composition of *Lactobacillus bulgaricus* is related to their resistance to freezing. Gilliland and Speck, 1974, working with cultures of lactic streptococci, found that the amount of octadecenoic acid present in the cells was inversely related to the cells' survival during freezing at  $-17^{\circ}\text{C}$ .

This investigation was undertaken to study the possible relationship of the fatty acid composition of various cultures of *K. fragilis* to their resistance to freezing at  $-19^{\circ}\text{C}$ .

## Experimental Procedure

Each strain of *K. fragilis* was grown in 300 ml of pepsinized whey (100 ml in each of three 250-ml Erlenmeyer flasks). The flasks were inoculated (1 percent) and incubated in a  $35^{\circ}\text{C}$  reciprocating shake water bath (82 strokes of 40 mm per min) for 15 hr. The cells were harvested by centrifugation and washed three times with 20 ml portions of cold sterile distilled water. The cells were finally resuspended in 8 ml cold sterile distilled  $\text{H}_2\text{O}$ . Half of the cell suspension was set aside in an ice-water bath for fatty acid analysis. Four ml of cold sterile 10 percent non-fat milk solids (NFMS) was mixed with

the remaining half and the resulting suspension frozen in 2 g aliquots at  $-19^{\circ}\text{C}$ . Survival of the yeasts during frozen storage was measured by determining colony counts for the suspensions before freezing and after one day of frozen storage at  $-19^{\circ}\text{C}$  using methods described previously by Bostian and Gilliland (1979.)

Free lipids were extracted from the washed yeast cells by a method similar to that of Bligh and Dyer (1959). The cells were partially hydrolyzed by adding 4 ml of 4 N HCl to 4 ml of the aqueous suspension of washed yeast cells and heating the mixture 15 min at  $121^{\circ}\text{C}$ . Ten ml chloroform and 20 ml methanol were added to the cooled mixture in a separatory funnel. The contents were shaken intermittently for 10 min. Following the extraction period, an additional 10 ml of chloroform and 10 ml distilled water were added to the funnel. The contents were shaken, the layers were allowed to separate, and the chloroform layer recovered. The chloroform was evaporated under  $\text{N}_2$  with slight heating to a volume of approximately 1 ml. Fatty acid methyl esters were prepared from the extracted lipid fractions using boron-trifluoride-methanol reagent by a method similar to that of Metcalfe *et al.* (1966). The methyl esters were stored at  $-20^{\circ}\text{C}$  until assayed.

The methyl esters were separated with an Aerograph Hy-Fi model 600 gas chromatograph (Wilkens Instrument and Research, Inc.) equipped with a hydrogen flame ionization detector. A 6-foot stainless steel column packed with DEGS (15 percent) on Gas-Chrom Q (100/120 mesh, Applied Science Laboratories Inc., State College, PA) was used for the analyses. The column oven temperature was regulated at  $180^{\circ}\text{C}$ , and the injector temperature was  $200^{\circ}\text{C}$ . Nitrogen was used as the carrier gas, flowing at the rate of 20 ml/min.

The identities of the fatty acid methyl esters were determined by comparing their retention times with those of known methyl esters. The peak areas were determined with a compensating polar planimeter (Keuffel and Esser Co.). The total peak area was used to establish the relative percent of each fatty acid present in the sample.

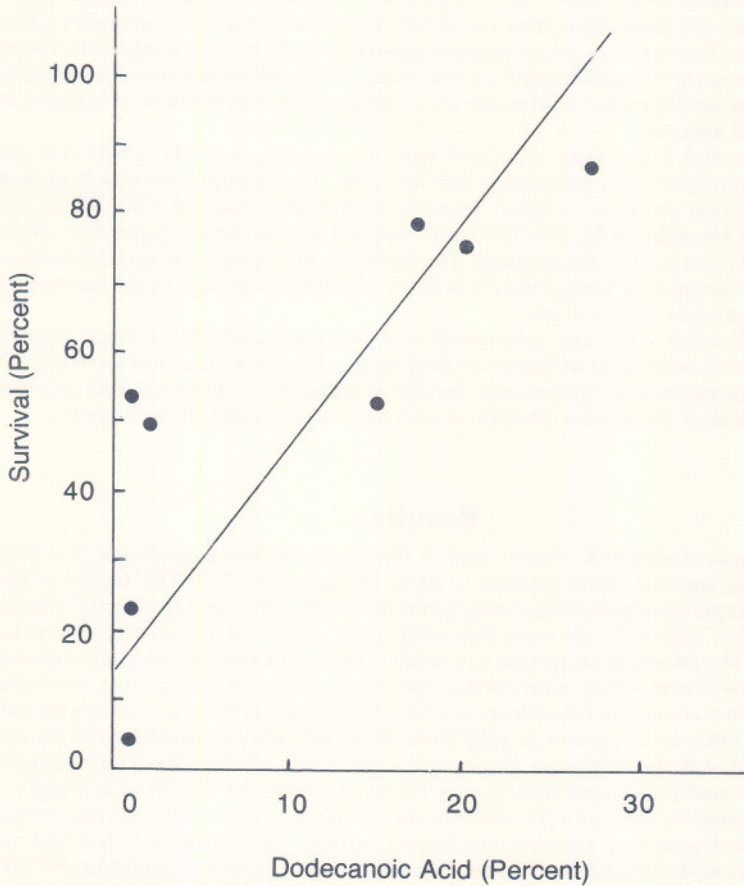
## Results

The eight strains of *K. fragilis* used in this study exhibited significant ( $P < .005$ ) variations in survival when exposed to 24-hr storage at  $-19^{\circ}\text{C}$ . The results of gas chromatographic analyses of the methyl esters from cells of the eight strains of *K. fragilis* indicated that essentially the same fatty acids were present in all strains of the yeast (Table 1). The following exceptions were noted. Two strains contained small amounts of octadecanoic acid (18:0), whereas the other strains contained none. Also, two other strains did not contain octadecadienoic acid (18:2), whereas the other six strains did. The other fatty acids present in cells from all strains were dodecanoic (12:0), tetradecanoic (14:0), hexadecanoic (16:0) and octadecenoic (18:1) acids. The amount of dodecanoic acid (12:0) appeared to vary the most among the strains of *K. fragilis*.

Regression coefficients were calculated for each fatty acid in relation to the percent survivors to determine if relationships existed between the resistance to freezing the cellular fatty acid composition. The only fatty acid which showed a significant ( $P < .05$ ) relationship to survival was dodecanoic acid. Figure 1 shows the regression of percentages of survivors on percent dodecanoic acid for the eight strains of *K. fragilis*. As the cellular content of this fatty acid increased, so did the ability of the yeast cultures to survive freezing at  $-19^{\circ}\text{C}$ . Selecting cultures having high levels of dodecanoic acid may be a means for choosing those best suited for preparing frozen concentrated cultures of this organism. Growth conditions might also be altered to favor the presence of this fatty acid in the cells, thus improving their resistance to freezing.

## Literature Cited

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**Figure 1. Regression of percent survival on percent of dodecanoic acid in cells of *K. fragilis*.**

**Table 1. Fatty acid composition of lipids from different strains of *Kluyveromyces fragilis*.**

Fatty acids <sup>a</sup>	Relative percentages							
	50-29 <sup>b</sup>	72-297	Y-1156	Y-1196	Y-108	Y-1171	50-16	C-106
12:0	27	20	1	15	17	2	1	1
14:0	6	4	6	4	2	5	5	7
16:0	29	34	31	40	40	38	25	36
? <sup>c</sup>	8	11	12	7	1	6	11	8
18:0	—	—	—	—	—	—	7	4
18:1	26	31	42	35	40	47	43	39
18:2	3	1	8	—	—	3	7	6

<sup>a</sup>Fatty acid methyl esters are designated by the number of carbon atoms to the left of the colon and the number of double bonds to the right.

<sup>b</sup>The numbers in this row indicate the identification number of the 8 strains of *K. fragilis*.

<sup>c</sup>The identity of this fatty acid was not determined because it did not appear to be related to the survival of the cultures.

## Percentage of Yeast Cells and Whey Protein in Material Recovered From Whey Cultured with *Kluyveromyces fragilis*

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Lactose in cheese whey can be completely utilized by growing *Kluyveromyces fragilis* in the whey. Removal of the resulting yeast cells and heat precipitated whey protein leaves a liquid much more acceptable than the original whey for disposal in a municipal waste system.

The yeast and heat-precipitated whey protein recovered from cheese whey can be used as a food ingredient. Considerable interest has also been shown for its use as a supplement for animal feeds. To have better knowledge of the material for such use, it would be desirable to know the relative amounts of yeast cells and whey protein it contains.

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