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The Effect of Freezing on Yeasts.

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The effect of freezing on yeasts has not received much study, at least under conditions which give information concerning their longevity, rate of death, etc. Doemens[1] exposed beer yeast to a temperature of about—190° C. for six minutes. It still retained its vitality. When he suspended the yeast in water and exposed to liquid air for 5 minutes and 20 minutes, and then thawed in cold water, its power of development was totally destroyed. Macfadyen and Rowland[2] subjected microorganisms, among which were yeasts, to a temperature of —252° C. for 6 months, after which the yeasts were reported to have suffered no reduction in vitality. They gave good growth and possessed unaltered powers of fermentation. One would infer from this report that yeasts are resistant to freezing and that there was no reduction in numbers. Bokorny[3] kept a sample of brewers' pressed yeast at a temperature of —15° C. for 24 hours and then allowed it to warm up slowly to a temperature of 7° C.; fermentation and reproduction went on for a short time but soon stopped. In another experiment the yeast was very quickly raised to 10° C.; after 4 weeks in a suitable medium it exhibited slight fermentation. Microscopic examination at this stage showed that most of the cells were dead and only a few were budding. Bokorny thus showed that beer yeast was resistant to cold but not as resistant as other microorganisms.

In the experimental work reported here 8 species of yeasts were used; 4 species of common bacteria were also included in order to act as controls since so much work has been done on the effect of freezing bacteria. *Escherichia coli, Serratia marcescens, Bacillus mesentericus* and *Bacillus subtilis, Saccharomyces cerevisiae, Saccharomyces ellipsoideus, Saccharomyces pastorianus, Saccharomyces marxianus, Pichia membranaefaciens, Zygosaccharomyces mongolicus, Torula rosea,* and *Mycoderma vini* were used. These organisms grew well on the ordinary laboratory media; the bacteria on plain media and the yeasts on glucose or malt media. The procedure for each of the organisms was as follows: 2 cc. of a 24 hour broth culture of the organism were transferred by means of a sterile syringe to each of 60 sterile vaccine ampoules which were then sealed immediately with a Bunsen flame. In like manner 60

TABLE I.

	Escherichia coli		Serratia marcescens		Bacillus mesentericus		Bacillus subtilis	
	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension
Initial count	18,500,000	212,000,000	24,000,000	145,200,000	2,100,000	17,600,000	1,850,000	15,000,000
Frozen 3 wks.			2,100,000	120,000,000				
" 4 wks.	2,000,000	6,000,000			1,200,000	15,000,000	1,200,000	9,500,000
" 5 wks.	1,200,000	4,650,000					1,500,000	7,000,000
" 6 wks.	50,000	3,000,000	100,000	110,000,000	1,100,000	13,200,000		
" 8 wks.	16,500	2,950,000	36,000	50,000,000	800,000	5,800,000	100,000	5,400,000
" 9 wks.	1,400	1,100,000	18,000	42,000,000	950,000	10,000,000		
" 10 wks.	300	1,230,000			600,000	7,000,000		
" 12 wks.	1,400	990,000	10,000	3,000,000	650,000	7,000,000	23,000	5,200,000
" 16 wks.	100	20,000	7,000	90,000	430,000	6,500,000		
" 18 wks.	40	18,000			500,000	6,000,000	13,000	5,100,000
" 20 wks.	1	15,000						
" 21 wks.	sterile	5,000						
" 22 wks.	sterile							
" 32 wks.			5,000	12,000				
" 34 wks.					250,000	4,000,000	14,000	4,500,000
" 38 wks.	sterile	sterile	100	10,000	130,000	3,400,000	11,000	5,100,000
" 80 wks.			360	100	23,000	2,500,000	2,000	200,000

TABLE II.

	Saccharomyces cerevisiae		Saccharomyces ellipsoideus		Saccharomyces pastorianus		Saccharomyces marxianus	
	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension
Initial count	300,000	6,900,000	1,050,000	3,750,000	1,600,000	4,000,000	30,000	3,500,000
Frozen 2 wks.					100,000	900,000	16,000	150,000
" 4 wks.	15,000	4,700,000	700,000	1,300,000	80,000	50,000		
" 5 wks.	10,300	4,400,000	1,200	170,000			7,000	39,000
" 6 wks.					55,000	64,000		
" 7 wks.	10,000	3,120,000	34	19,000	50,000	53,000	4,200	19,000
" 8 wks.			20	10,000	38,000	42,500		
" 9 wks.	8,800	2,000,000	17	11,500				
" 10 wks.	5,500	1,900,000	16	10,000	40,000	38,000	5,300	6,400
" 12 wks.	7,000	400,000	10	12,000	18,000	40,000	6,000	7,000
" 14 wks.	6,500	190,000	6		10,000	30,000	4,500	3,800
" 16 wks.			4	8,000			3,000	4,000
" 18 wks.			7	3,000				
" 22 wks.			5	600				
" 30 wks.	3,000	32,000			6,500	30,000	1,700	3,400
" 38 wks.			1	400				
" 58 wks.	2,000	64,000	1	40	1,300	30,000	300	1,000
" 66 wks.	2,300	60,000	3	200	3,000	30,000	600	5,000
" 80 wks.	1,500	65,000	0	250	2,400	21,000	400	4,000
" 160 wks.	950	53,000	0	95	1,600	19,000	120	2,200

TABLE III.

	Pichia membranaefaciens		Torula rosea		Zygosaccharomyces mongolicus		Mycoderma vini	
	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension	Broth Culture	Physiological Sodium Chloride Suspension
Initial count	70,000	3,300,000	4,300,000	5,000,000	24,000,000	2,000,000	1,800,000	48,000,000
Frozen 2 wks.	20,000	200,000	100,000	3,300,000			1,300,000	38,000,000
" 4 wks.	1,000	50,000	60,000	700,000	3,000,000	800,000		
" 5 wks.	900	11,000	10,000	290,000			1,000,000	41,000,000
" 7 wks.	13,000	8,000	7,000	130,000	800,000	950,000	850,000	27,000,000
" 9 wks.	500	1,000	5,000	20,000	100,000	130,000	600,000	18,000,000
" 10 wks.			3,000	20,000	150,000	200,000		
" 26 wks.	sterile	sterile	160	1,500	10,000	60,000	150,000	5,000,000
" 46 wks.	sterile	sterile	1	500	200	20,000	8,000	260,000
" 54 wks.	sterile	sterile	2	2	500	1,000	500	110,000
" 68 wks.	sterile	sterile	3	1	200	800	100	60,000
" 160 wks.			sterile	sterile	3	2	20	2,000

sterile ampoules were filled with 2 cc. of a suspension of these organisms in physiological NaCl solution. These suspensions were prepared from 24 hour agar slant cultures and shaken thoroughly on a shaking machine to insure an even suspension. Plate counts were made of the broth culture and of the suspension to determine the initial count before freezing. The ampoules were then placed in the trays of an electric refrigerator of the Frigidaire type at a temperature ranging from —13° to —15° C. This temperature was maintained constantly throughout the experiment except for one day when the temperature was a little above zero. The ampoules were removed at regular intervals, allowed to melt at room temperature, shaken thoroughly, and 1 cc. of the contents plated in different dilutions to determine the change in the count if any had occurred.

The accompanying tables summarize the results of the experiment, giving the initial counts of the broth culture and of the suspensions, and the counts obtained after the cultures have been frozen for various lengths of time. Table I is a record of the bacterial counts; Tables II and III are records of yeast counts.

These data indicate that the number of living cells in suspensions of bacteria and yeasts undergoes a rapid reduction at first; after a longer period of time the reduction is very slow. Non-spore-forming bacteria were much more susceptible to the harmful effects of freezing than the spore-forming species. A great variation in resistance to temperatures below freezing was noticed among the yeasts which were used.

Conclusions: 1. The prolonged action of freezing temperatures destroys the cells of yeasts and bacteria. 2. The rate of death follows the curve of a monomolecular reaction, the death rate being proportional to the number of living cells. 3. There is a great variation among yeasts and bacteria in their resistance to freezing. 4. Under the conditions of this experiment, the death rate seemed to be slower in the physiological sodium chloride solution than in the broth.

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[1] Doemans, reviewed in *J. Fed. Inst. Brew.*, 1901, vii, 299.

[2] Macfadyen, A., and Rowland, S., *Ann. Bot.*, 1902, xvi, 589.

[3] Bokorny, M., *Brau. u. Hopfen-Zeit.*, 1927, cxli; reviewed in *J. Inst. Brew.*, 1927, xxxiii, 520.