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Influence of Alcoholic Fermentation Temperature on Sorghum Beer Quality

Kouadio Florent N'Guessan[1*] • N'dédé Théodore Djeni[1] • Adjéhi Thomas Dadie[1,2] • Koffi Marcellin Dje[1]

[1] Laboratoire de Microbiologie et Biologie Moléculaire, Unité de Formation et de Recherche en Sciences et Technologie des Aliments (UFR-STA), Université d'Abobo-Adjamé, 02 BP 801 Abidjan 02, Ivory Coast
[2] Institut Pasteur de Côte d'Ivoire, 01 BP 490 Abidjan 01, Ivory Coast
Corresponding author: * genie_ci@yahoo.fr

ABSTRACT

The influence of fermentation temperature (variable from ambient to 40°C) on yeast population and some physico-chemical characteristics was studied during sorghum beer processing. Gas release started quickly at 35 and 40°C but the production rate was higher at 35°C than at other temperatures and reached its maximum value at the same time (after 5 h) for fermentations at 35 and 40°C. Maximum sugar consumption rate occurred during the first four hours of fermentation for all temperatures, except at ambient but was slightly more elevated at 35°C. This led to higher ethanol production at 35°C than at other temperatures even though the coefficient of sugar conversion into ethanol was similar for all four fermentation systems. Thus, the beer produced at 35°C contained the lowest sugars and the highest alcohol. In contrast, organic acid content was not influenced by temperature. However, yeast growth rate decreased at high temperatures and the declination phase which occurred after 8 h of fermentation was highest at 35°C.

Keywords: ethanol, gas released, organic acids, sugars, traditional starter, yeast

INTRODUCTION

Sorghum beer is a traditional alcoholic beverage in sub-Saharan Africa. But depending on the geographical location it is designated under different product names (van der Aa Kühle *et al*. 2001). In Ivory Coast, it is called ''tchapalo'' (Yao *et al*. 1995). The brewing of sorghum beer involves malting, souring, boiling, mashing, straining and alcoholic fermentation (Haggbade and Holzapfel 1989; Nanadoum Maoura *et al*. 2006; Sawadogo-Lingani *et al*. 2007), in which variations may occur depending on the regional location (van der Aa Kühle *et al*. 2001). Alcoholic fermentation is the final step in the production of sorghum beer and is usually initiated by pitching the sweet wort with either a portion of previous brew or dried yeast harvested from such (Yao *et al*. 1995; Nanadoum 2001).

Several factors, such as composition of the final wort, brix value, yeast involved, starter rate and fermentation temperature could strongly affect alcoholic fermentation, and, as a consequence, the quality of the beer. In order to improve fermentation and produce consistent and high-quality final products, process control of these parameters is required. One of these factors, the temperature of fermentation, mainly affects: (i) the growth of the yeasts and therefore the duration of the fermentation, (ii) the contribution of different yeast species on fermentation and (iii) the metabolism of the yeasts (Lafon-Lafourcade 1983; Fleet and Heard 1993). According to temperature, it was defined, among yeast belonging to the *Saccharomyces sensu stricto* complex, cryotolerant strains (*S. bayanus* and *S. pastorianus*) and non-cryotolerant strains (*S. cerevisiae* and *S. paradoxus*) (Guidici *et al*. 1998). Cryotolerant strains are well known in brewing and oenology and possess a number of advantages compared to non-cryotolerant strains (Serra *et al*. 2005).

Sorghum beer production is traditionally carried out under ambient conditions which vary from day-to-day and according to the season. Moreover, fermentation begins at the sweet wort temperature which is suitable for each brewer (Djè, pers. comm.). Thus the determination of optimal operating temperature could be very useful.

The aim of the present work was to study the impact of different fermentation temperatures on the amount of ethanol, residual sugars and organic acids in sorghum beer.

MATERIALS AND METHODS

Fermentation experiment

The fermentation medium was made from sweet wort (Aka *et al*. 2008) and dried yeasts. The sweet wort was obtained from randomly identified commercial *tchapalo* brewer at Williamsville-Macaci and dried yeasts from randomly identified commercial *tchapalo* brewers at Abobo-Soghefia, Williamsville-Macaci and Cocody-Blockosso in the district of Abidjan, Southern Ivory Coast.

Fermentations were performed in 500 ml Erlenmeyer flasks filled with 400 mL of sweet wort and inoculated with dried yeasts at 0.8% (w/v). The flasks were covered with a cotton cap and incubated for 12 h at four different temperatures [30, 35, and 40°C, and ambient temperature (AT)] without shaking.

Fermentations were repeated three times and samples were taken at 0, 4, 8 and 12 h from fermenting wort.

Gas released from fermenting wort

The volume of gas produced during fermentation was measured using an experimental device described by Pol (1996). Fermentation flasks filled with 200 mL of sweet wort and inoculated at 0.8% (w/v) of dried yeasts were incubated for 12 h at four different temperatures [30, 35, and 40°C, and ambient temperature (AT)] without shaking. The gas released was measured as quantity (mL) of displaced water per hour. Measures were repeated three times.

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Original Research Paper

Determination of sugars in the fermenting wort

The total soluble solids (TSS) content, expressed as °Brix value, were determined in each sample using a hand refractometer and water-soluble carbohydrates were determined by the phenol sulphuric acid method, according to Dubois *et al.* (1956).

Ethanol content

Ethanol was determined by gas-chromatographic analyses, carried out using a Shimadzu CG-14A gas chromatograph. Fermenting wort samples (2 µL) were filtered and injected directly. The temperature was set at 8°C/min. Injector and detector temperatures were 200 and 250°C, respectively. Helium at 2 Kg/cm^2 was used as the carrier gas and the external standard method was used for the quantitative determination of ethanol.

Organic acids

Sample preparation for HPLC

Samples were firstly centrifuged at 3000 rpm for 20 min. Then, they were filtered through a 0.45 µm Millipore membrane filter (Sartorius AG, Goëttingen, Germany) and stored at -20°C until analyses.

HPLC equipment and operating conditions

The HPLC system (Shimadzu Corp., Japan) was equipped with a pump (Shimadzu LC-6A Liquid Chromatograph), a detector (Shimadzu SPD-6A UV Spectrophotometric detector) and an Integrator (Shimadzu C-R 6A Chromatopac). Chromatographic separation was performed using an ion-exclusion ORH-801 column (300 × 6.5 mm, Interchrom, France). The eluant was 0.004 N H_2SO_4 with a flow rate of 0.8 mL/min and the detector was set at 210 nm.

A 20 µL injection volume was used for HPLC samples. Analyses were done in duplicate and mean values were used.

The organic acid standards were diluted in distilled water at concentrations ranging from 0.05-0.4 g/L. The standards were filtered and injected separately.

Components were identified and quantified by comparison of their retention times and peak areas with those of standards.

Microbiological analysis

The fermenting wort samples were directly diluted in ten-fold series in Buffered Peptoned Water (BIO-RAD, France) and aliquots (0.1 ml) were plated in duplicate on Sabouraud Chloramphenicol agar medium (BIO-RAD, France). After incubation at 30°C for 3-5 days, yeasts were enumerated on plates displaying 30 to 300 colonies and results were expressed as Log (cfu/mL) of sample.

Statistical analysis

Analysis of variance (ANOVA) and Duncan's Multiple Range Test were carried out with the software Statistica, 1999 Edition for each fermentation parameter and sorghum beer compound to test differences among temperature on fermentation capability and beer quality. Statistical differences with P-values under 0.05 were considered significant.

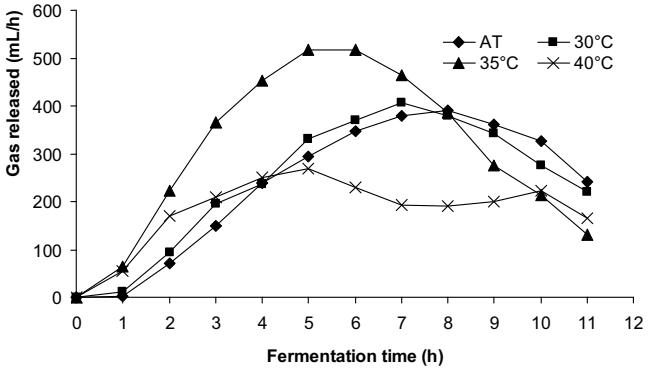

Fig. 1 Effect of temperature on gas released during alcoholic fermentation of sorghum beer.

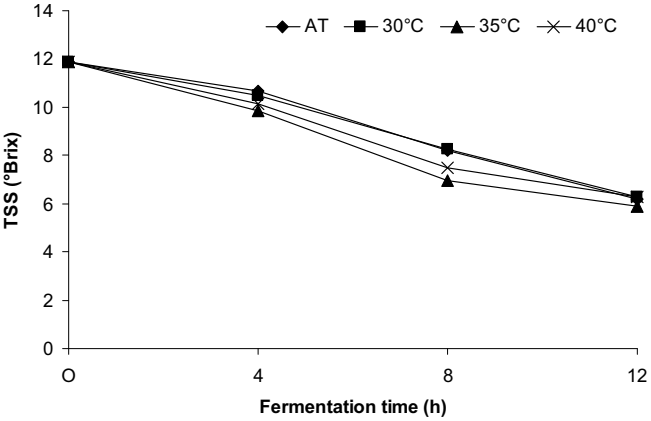

Fig. 2 Total soluble solids (TSS) depletion during alcoholic fermentation of sorghum beer at different temperatures.

RESULTS

Gas released

Fig. 1 shows gas released during fermentation. Gas production started quickly in trials fermented at 35 and 40°C. But after 1 h of fermentation, the system at 35°C showed the highest production rate, reaching a maximum value of 516.7 mL/h after 5 h. The system at 40°C also reached its maximum gas production rate (268.7 mL/h) after 5 h of fermentation.

Gas production at AT and 30°C was similar. It started slowly and reached a maximum of 406.7 mL/h after 7 h at 30°C and 390 mL/h after 8 h at AT.

At 35°C, the gas curve presented a series of short-lag, exponential, stationary and decline phases of production, whereas at the other temperatures no stationary phase was observed.

The total volume of gas produced after 11 h of fermentation was 2.8, 2.86, 3.61 and 2.16 L for the system at AT, 30, 35 and 40°C, respectively.

Sugar content

Fig. 2 shows that the depletion of TSS present in the wort was higher at 35°C than at other temperatures. AT and 30°C

Table 1 Fermentation kinetics of sorghum beer carried out at different temperatures.

	AT	30°C	35°C	40°C
Residual sugars (g L^{-1})	28.07 ± 16.35	27.36 ± 10.53	22.48 ± 14.20	25.95 ± 14.54
Sugar consumed (%)	63.64 ± 21.52	62.01 ± 19.21	70.21 ± 19.21	67.94 ± 17.36
q_s^{max} (g L^{-1} h^{-1})	5.38 (4-8 h)	5.24 (0-4 h)	7.72 (0-4 h)	7.82 (0-4 h)
q_s (g L^{-1} h^{-1})	4.51 ± 0.68	4.57 ± 0.53	4.98 ± 2.54	4.69 ± 2.44
C	0.051 ± 0.021	0.048 ± 0.002	0.049 ± 0.013	0.049 ± 0.012

Values ± standard deviations are average of three independent trials
q_s^{max}, q_s, maximum and average sugar consumption rate respectively
C, coefficient sugar-ethanol conversion (% Vol. ethanol produced/ % w sugar consumed)

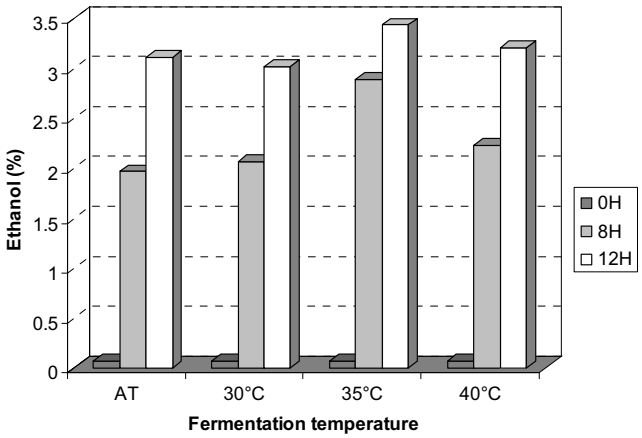

Fig. 3 Temperature effect on the production of ethanol during alcoholic fermentation of sorghum beer.

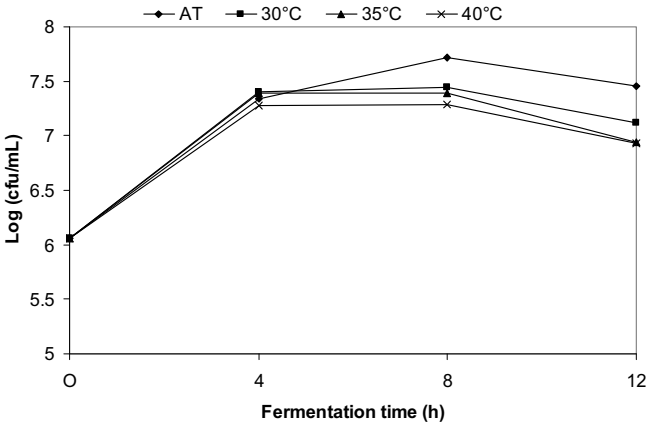

Fig. 4 Temperature effect on the yeast growth during alcoholic fermentation of sorghum beer.

showed a similar depletion of TSS, although differences were not significant (P> 0.05) between all temperatures during fermentation.

In the final beer produced, the sugar content ranged between 22.48 and 28.07 g/L (**Table 1**). Microorganisms at 35°C had the highest capacity to use sugars and they metabolised 70.21% of the sugar present in the wort with an average sugar consumption rate of 4.98 g/L/h. The maximum sugar consumption rate (7.72 g/L/h) was observed between 0 and 4 h of fermentation. Nevertheless, results on the coefficient of sugar conversion to ethanol were similar for the four fermentation systems.

Ethanol production

The rate of alcoholic fermentation at different temperatures is shown in **Fig. 3**, measured by ethanol production. Beers produced with controlled temperatures showed higher levels of ethanol than that fermented at AT. The ethanol content after 12 h of fermentation varied from 3.02 to 3.43%. Fermentation at 35°C showed the highest content of ethanol although values were not significantly different (P> 0.05).

Organic acids during fermentation

Table 2 illustrates the time course of organic acids in fermenting sorghum wort at various temperatures. According to these data, fluctuations in organic acids were not regular for each compound regardless of temperature and these fluctuations were very slight. Indeed, lactic acid contents were respectively 12.82, 12.38, 12.59 and 13.61 g/L in beers produced at AT, 30, 35 and 40°C. In the same way, tartaric acid values were 1.52, 1.36, 1.46 and 1.61 g/L. Only malic acid content at 35°C after 12 h of fermentation was significantly different (0.99 g/L). Lactic acid represented 61 to 68% of total organic acid analyzed. In the beers produced, the tartaric, lactic, fumaric and propionic acid contents were slightly high at 40°C. Besides, the highest amount of total organic acids after 8 h (21.08 g/L) and 12 h (21.54 g/L) of fermentation were obtained respectively at 30 and 40°C.

Growth and survival of yeast during fermentation

Temperatures grouped in two different profiles of growth (**Fig. 4**). In fermentation systems at 30, 35 and 40°C, yeast cells reached a maximum cell concentration of 7.27–7.4 log (cfu/mL) after 4 h of fermentation. Each fermentation system maintained this cell level until 8 h of fermentation, after

Table 2 Organic acids profile of fermenting sorghum wort during beer production under different temperatures.

	Time (h)	Atmospheric Temperature	30°C	35°C	40°C
Oxalic acid (g/L)	0	0.55 ± 0.23	0.55 ± 0.23	0.55 ± 0.23	0.55 ± 0.23
	8	0.50 ± 0.15	0.54 ± 0.15	0.50 ± 0.14	0.51 ± 0.15
	12	0.60 ± 0.15	0.49 ± 0.12	0.54 ± 0.1	0.52 ± 0.17
Citric acid (g/L)	0	0.46 ± 0.36	0.46 ± 0.36	0.46 ± 0.36	0.46 ± 0.36
	8	0.40 ± 0.48	0.40 ± 0.08	0.49 ± 0.31	0.40 ± 0.1
	12	0.27 ± 0.23	0.34 ± 0.07	0.43 ± 0.08	0.28 ± 0.27
Tartaric acid (g/L)	0	1.11 ± 0.71	1.11 ± 0.71	1.11 ± 0.71	1.11 ± 0.71
	8	1.69 ± 0.92	1.45 ± 0.19	1.58 ± 0.76	1.48 ± 0.36
	12	1.52 ± 0.31	1.36 ± 0.48	1.41 ± 0.56	1.61 ± 0.45
Malic acid (g/L)	0	1.03 ± 0.76	1.03 ± 0.76	1.03 ± 0.76	1.03 ± 0.76
	8	0.34 ± 0	0.67 ± 0.31	0.52 ± 0.09	0.56 ± 0.15
	12	0.54 ± 0.05	0.58 ± 0.14	0.99 ± 0.13	0.43 ± 0
Lactic acid (g/L)	0	13.14 ± 2.81	13.14 ± 2.81	13.14 ± 2.81	13.14 ± 2.81
	8	11.60 ± 1.52	13.13 ± 3.13	11.13 ± 1.95	12.25 ± 1.58
	12	12.82 ± 0.44	12.38 ± 0.36	12.59 ± 0.32	13.61 ± 3.58
Fumaric acid (mg/L)	0	14.19 ± 6.76	14.19 ± 6.76	14.19 ± 6.76	14.19 ± 6.76
	8	14.82 ± 8.50	13.79 ± 4.70	9.39 ± 4.53	23.07 ± 15.88
	12	16.00 ± 0.69	11.07 ± 9.91	12.43 ± 2.79	17.78 ± 6.79
Propionic acid (g/L)	0	2.97 ± 1.42	2.97 ± 1.42	2.97 ± 1.42	2.97 ± 1.42
	8	3.51 ± 1.41	4.85 ± 2.12	3.35 ± 2.42	3.08 ± 0.69
	12	3.15 ± 1.31	5.00 ± 3.13	3.20 ± 0.90	5.05 ± 4.16
Total organic acids (g/L)	0	19.30 ± 5.19	19.30 ± 5.19	19.30 ± 5.19	19.30 ± 5.19
	8	18.09 ± 3.53	21.08 ± 5.15	17.60 ± 1.98	18.30 ± 1 2.03
	12	18.93 ± 1.25	20.18 ± 2.75	19.17 ± 1.42	21.54 ± 1.91

Values ± standard deviations are average of three independent trials.

which cells slowly died off, reaching 7.11, 6.94 and 6.92 log (cfu/mL) respectively at 30, 35 and 40°C. Cell death was greater at 35°C than at other temperatures. Moreover, cell growth decreased with an increase in temperature.

In the fermentation system at AT, yeast cells reached a maximum cell concentration of 7.71 log (cfu/mL) after 8 h of fermentation. Subsequently, yeast cells died off, reaching 7.45 log (cfu/mL) after 12 h of fermentation.

DISCUSSION

Temperature is known to affect yeast metabolism and, as a result, the formation of secondary metabolites (Lafon-Lafourcade 1983) and the quality of the final product. In this work we intended to use a simple experimental approach to study its effect on sorghum beer produced in the Ivory Coast. Several fermentation parameters such as changes in sugars and yeast population were assayed.

As expected, gas released by yeast during the alcoholic fermentation varied according temperature. During the lag phase of gas production, yeast cells in the wort adjust their metabolism in order to take advantage of the new environmental and initiate exponential growth (Buchanan and Klawitter 1991). Moreover, during this phase a pseudo-aerobic fermentation process occurs and most of the acetaldehyde is synthesized (Ribéreau-Gayon *et al*. 1976; Martinez *et al*. 1997). The lag time was shorter at 35 and 40°C (less than 1 h) than at AT and 30°C (1 h). These results obtained indicate that the lag phase duration increases with decreasing incubation temperatures. Similar observations were seen in a study by Buchanan and Klawitter (1992), who measured the lag phase duration for *Escherichia coli* O157:H7. The exponential phase was higher and shorter at 35°C, which means that yeasts involved in sorghum beer production were most active at this temperature.

The decline phase was observed after 5 h at 40°C, 6 h at 35°C, 7 h at 30°C and 8 h at AT. This might indicate that temperature affected the duration of fermentation and the metabolism of yeasts (Lafon-Lafourcade 1983; Fleet and Heard 1993).

The speed of gas production during the fermentation increased with temperature. But over 35°C, this speed fell. Therefore, 35°C seemed to be the optimal temperature of gas production for yeasts implied in the alcoholic fermentation of sorghum wort. In addition, after gas production maximal value followed a speed reduction which was more important at 35°C than at the other temperatures. Our results demonstrated that the decline phase was closely related to the decrease of sugars consumption and yeast cell concentration.

Like gas production, the depletion of sugars was similar at AT and 30°C, and more important at 35°C. The drop in sugar levels was presumably a result of their being utilized as carbon and energy sources. This higher yeast activity at 35°C was demonstrated by the most elevated percentage of sugar consumed (70.21%) and the lowest content of sugar that remained (22.48 g/L). Besides, the average sugar consumption rate was more elevated at 35°C. Our results show that beer produced at 35°C is least sugary, unlike that produced at AT. The transport of sugar in yeast cells occurs by facilitated diffusion, and membrane fluidity would therefore be more important at 35°C than at other temperatures. These results are in agreement with findings in which temperature affects the fluidity of the membrane (Leão and van Uden 1985; Llaurado 2002). So, low temperatures reduce the fluidity of cell membrane and restrict structure changes while high temperatures cause some opposite effects. Banat *et al*. (1998), however, reported that the efficiency of *Saccharomyces cerevisiae* fermentation over 35°C is weak because of the high fluidity of membranes. The maximum sugar consumption rate was observed most of the time at the beginning of fermentation (0–4 h) and increased with temperature. Therefore temperature might not be the only factor which influenced the speed of sugar consumption. It was reported that ethanol influences sugar transport yield and exercises a direct inhibition (Leão and van Uden 1985; Llaurado 2002). In the same way, an increase of membrane fluidity was observed in the presence of ethanol (Alexandre *et al*. 1994). Changing the temperatures clearly improved the production rate of ethanol. Although the coefficient of sugar-ethanol conversion was more elevated at AT, 35°C presented the most elevated rate of ethanol during fermentation. The high sugar consumption speed at this temperature could explain this fact. Over 35°C, ethanol production decreased. So fermentation over 35°C favoured a high glyceropyruvic fermentation products formation and a minor ethanol production (Torija 2002). As a consequence, 35°C was the optimal temperature of ethanol production for yeasts in the traditional starter. However the low production observed at 8 to 12 h fermentation was linked to the poisonous effect of ethanol on yeasts. These results are in agreement with those of several authors who underlined that in presence of ethanol, an exponential cellular mortality induction was observed in a range of temperature from 25 to 40°C (van Uden 1985; Sá-Correia and van Uden 1986). In addition, Barre *et al*. (1998) reported that high ethanol content lead to a faster mortality while Boulton *et al*. (1996) showed that ethanol inhibitory effect depends strongly on temperature.

Neither yeasts, nor lactic acid bacteria present in the fermenting wort (Nanadoum Maoura *et al*. 2006) produced or consumed high quantity of organic acids. This is in accordance with the studies of Aka *et al*. (2008). There were only slight fluctuations of these acids. Fermentation at 40°C gave slightly more elevated organic acid contents by the way of glyceropyruvic fermentation which is favoured at temperatures over 35°C (Torija 2002).

The growth of yeasts permitted us to observe growth curves with an exponential, stationary and decline phases at 30, 35 and 40°C, respectively. During the exponential phase yeast viability decreases as temperature increases (Ough 1966; Nagodawithana *et al*. 1974; Casey *et al*. 1984). This decrease was thought to be due to a greater accumulation of intracellular ethanol at higher temperatures, which would produce cell toxicity (Nagodawithana *et al*. 1974) and would alter the structure of the membrane, decreasing its functionality (Lucero *et al*. 2000). Ethanol tolerance of some yeast species depends on the temperature (Casey and Ingledew 1986; D'Amore and Stewart 1987; Gao and Fleet 1988). At AT, yeast growth increased until 8 h before decreasing because of low ethanol content and temperature.

The yield of ethanol and other sorghum beer compounds are related to temperature during alcoholic fermentation. The highest microbial and physico-chemical activities were obtained at 35°C. Temperature of fermentation could then clearly affect sorghum beer quality. As a consequence, the dynamics of yeast strains population and their endurance to different temperatures could be affected. Thus, temperature could be a criterion for indigenous microbiota selection for further purposes such as selecting fermentation starters.

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