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Research Article

Establishment of a Sterilization Regime for Sugarcane Molasses Used in Baker's Yeast Production

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Abstract

Background and Objective: *Geobacillus stearothermophilus* is a major contaminant of sugarcane molasses which withstand the traditional primary heat treatment of crude molasses and compete with baker's yeast in sugar fermentation. Present work aims to study the thermal death kinetics of *G. stearothermophilus* and to establish a heat treatment regime for the molasses used in baker's yeast production. **Methodology:** Thermal inactivation studies were carried out on spores of *G. stearothermophilus* strain-isolated from Egyptian sugarcane molasses and populated on TSB as well as diluted molasses (10° Brix) adjusted to pH 4.7. Thermal death kinetics were calculated using TDT-tubes at temperatures ranging from 90-130°C. The thermal death experiments were done after 1 h from primary preheating the spore suspensions at 80°C for 10 min to eliminate the vegetative cells and induce heat shock for the spores. **Results:** Survival curves of spores are linear following first-order kinetic pattern for spore heated at temperatures >115°C. Obtained decimal reduction time (D-values) were 23.71, 8.07, 4.68, 0.834, 0.473 and 0.174 min for spore suspensions heated in molasses media at 100, 110, 115, 120, 125 and 130°C, respectively. Spore suspensions heated in TSB media showed higher D-values. The temperature range (Z-value) required to reduce the D-value by 1/10 was in the range of 14.05°C. The obtained D and Z-values were used to establish a continuous sterilization regime for molasses to achieve a reduction of *G. stearothermophilus* spores of 7 logarithmic cycles to insure molasses sterility. The proposed sterilization system is based on steam injection, holding the molasses in a sterilization tube for the necessary time and flashing to reduce the molasses temperature. Calculated sterilization time was 73.08, 32.11 and 14.11 sec for sterilization temperatures 130, 135 and 140°C, respectively. The corresponding length of necessary holding tube was 89.16, 39.17 and 17.21 m, respectively. **Conclusion:** The pump power required to force the flow of molasses through sterilization system was in the range of 1.37-1.7 HP and the steam pressure needed for sterilization was 4-5 bar. The steam requirement is of 1 kg steam for each 10 kg molasses.

Key words: Baker's yeast, *G. stearothermophilus*, thermal death kinetics, continuous sterilization

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Baker's yeast industry is one of the oldest and important industries in the world and also in Egypt. Molasses from sugar industry is the substrate material used worldwide for their population and production because of its content an fermentable sugars and other nutrients necessary for the growth and production of yeast. To assure safety of the produced baker's yeast on industrial scale, molasses was diluted to 40% Brix, acidified with phosphoric acid, heated by direct steam injection to 90-95 °C, agitated for 2 h and the clarified molasses was separated, cooled to 40-50 °C, further diluted and pumped to fermentation tanks.

The applied heat treatment may be sufficient to precipitate the suspended materials in molasses and to kill pathogenic and mesophilic bacteria. However, thermophilic bacteria and spores survive this heat treatment, compete with the yeast during the fermentation process, decreases the fermentation power of the produced yeast during bread manufacture and contaminates the baker's yeast.

Microbiological studies revealed the contamination of molasses and the final process yeast product with other species of microorganisms. Pillai *et al.*¹ studies the microbial diversity in solid waste molasses of sugar industry and they isolated five species of bacteria, six species of fungi belonging to genus *A. niger*, *A. terreus*, *A. flavus*, *Penicillium* sp., *Fusarium* sp. and *Rhizopus* sp.

Nolasco Junior and De Massaguer² stated that sugarcane molasses sustain a diversified microbioata predominantly formed by Gram positive bacteria such as *Lactobacillus* sp. and *Bacillus* sp., including the heat-resistance spore former *Bacillus stearothermophilus*, which makes out 6.9% of the contaminant microflora. Recently, Salem *et al.*³ examined the microbial profile of compressed baker's yeast produced in Egypt and found that the count of contaminating bacteria ranged between 7.44-8.24 log CFU g⁻¹ indicating that it is a major constituent of baker's yeast. The thermophilic spore former *Bacillus stearothermophilus* showed antimicrobial activity against baker's yeast (*Saccharomyces cerevisiae*).

The modern baker's yeast industry in the world has added additional heat treatment at higher temperature (T>130 °C) for the clarified molasses to insure the inactivation of *B. stearothermophilus* spores before feeding to fermentation tanks⁴. Lciek *et al.*⁵ mentioned that it is necessary to know both thermal inactivation of microorganisms that are present in the medium and the parameters which determine their heat resistance. These parameters are essential for the correct determination of proper conditions for thermal processing and product

sterility. According to Quantz⁶, the loss in sugarcane molasses at pH 4.7-5 could be negligible during the sterilization process.

Therefore, the purpose of the present work was to study the kinetics of thermal death of *Geobacillus* (formaly *Bacillus*) *stearothermophilus* isolated from Egyptian sugarcane molasses as well as to establish an additional heat treatment regime for the molasses before feeding to fermentation tanks under the conditions of baker's yeast production in Egypt.

MATERIALS AND METHODS

Definition and source of target microorganisms: The target microorganisms is *G. stearothermophilus* was originally isolated from Egyptian molasses as reported by Salem *et al.*³. The microorganism was identified using 16S rDNA sequencing and it was deposited into the GenBank as *Geobacillus stearothermophilus* NS4182-05 with accession number KP 243199.

Spore production of *G. stearothermophilus*: The methods of Stumbo⁷ and Lciek *et al.*⁵ were applied to obtain the spores of *G. stearothermophilus*. The *G. stearothermophilus* culture was grown on pre-sporulating liquid Tryptic Soya Broth (TSB) medium (LAB M, UK) for 18-20 h at 55 °C. Bacterial spores were then obtained in a surface culture on petri dishes 15 cm in diameter containing sporulating solid nutrient agar (bio world, USA). The inoculated dishes were incubated for 48 h at 55 °C. Bacterial biomass was washed from dish surface with sterile distilled water to obtain a suspension of spores of approximately (10⁸ mL⁻¹). The spore suspension in water was stored at 4 °C until needed. According to Wescolt *et al.*⁸, the stock spore suspension could be stored at 4 °C for 7 months without any adverse effects.

Spore activation: Aqueous suspensions of spores were preheated at the temperature 80 °C for 10 min to eliminate vegetative cells from the bacterial spore suspension and to induce heat shock to the spores, preventing shoulders on survivor curves. The procedure was carried out according to Feeherry *et al.*⁹, Lciek *et al.*⁵, Nolasco Junior and De Massaguer² and Dlugokenski *et al.*¹⁰ within 1 h prior to each experiment of thermal death determination.

Heating media: Viability of *G. stearothermophilus* spores was tested in TSB medium as well as in diluted molasses (Brix 10°) acidified with phosphoric acid using Thermal Death Time (TDT) tube method. The pH value of TSB medium was 7, while that of molasses was 4.7.

Thermal treatment: Thermal death kinetics of *G. stearothermophilus* NS4182-05 were carried out by the sealed tube method used for Thermal Death Time (TDT) determination according to Stumbo⁷. The thermal death time tubes of 75 mm length, 6 mm inside diameter and 0.2 mm glass thickness were sterilized for 2 h at 180°C dry heat before use. For thermal death experiments, the tube received 0.2 mL of spore suspension with an average initial population of 10⁸ spores mL⁻¹ and filled up with 1.8 mL either by tryptic soya broth or diluted molasses and sealed. The tubes were then submerged in thermostatic bath using water (for T < 95°C) and glycerol (for T > 95°C). The applied temperatures were 90, 100, 110, 115, 120, 125 and 130°C. At definite time intervals relative to each temperature, the thermal death time tubes were taken out and cooled immediately in ice bath.

The survivor count were obtained using decimal serial dilution in 0.1% peptone water and pour plating technique on nutrient agar media and incubated at 55°C for 48 h. After incubation period, the results were obtained and expressed as colony forming unit per milliliter (CFU mL⁻¹). The thermal lag time (time in center of the tubes) for each applied temperature was determined in TDT-tubes holding 2 mL sugarcane molasses using a thermocouple located at the center of the tube. The come-up time ranged between 22-55 sec for the temperature range 90-130°C and it was taken in consideration during calculation of death kinetic parameters.

The come-up time obtained in the present study agrees with those reported by Wang *et al.*¹¹, Yokoya and York¹² as well as Abraham *et al.*¹³.

Evaluation of survivor curves: Thermal inactivation kinetics of microorganisms is obtained by first establishing survivor curve, which is a plot of the number of microorganisms surviving a given heat treatment at a constant temperature against the heating time. According to Wang *et al.*¹¹, Feeherry *et al.*⁹, Abraham *et al.*¹³ and Iciek *et al.*⁵, the survivor curve of *G. stearothermophilus* follow a linear reaction order as follows in Eq. 1:

$$\ln N = \ln N_0 - k.t \quad (1)$$

Where:

- N₀ = Initial number of microorganisms (MO)
- N = Number of MO at any time during heating treatment
- k = Thermal death rate (min⁻¹)
- t = Heating time (min)

The obtained K-values could be used to calculate the decimal reduction time (D-value) which is defined as the time (in minutes or in seconds) to reduce the population by 90% at a constant lethal temperature as follows in Eq. 2:

$$D = 2.3/k \text{ (min)} \quad (2)$$

According to Abraham *et al.*¹³, the treatment time should be chosen to ensure spore destruction at least equivalent to two decimal reductions.

The temperature dependence of K and D is based on the assumption that the thermal death time (D-value) of microorganisms follows a semi-logarithmic relationship with temperature as follows¹⁴ in Eq. 3:

$$\log \frac{D_1}{D_2} = \frac{T_2 - T_1}{Z} \quad (3)$$

Where:

- D₁ = Decimal reduction time at T₁
- D₂ = Decimal reduction time at T₂
- Z = Temperature range required to reduce D-value by 1/10

The dependence of K-value on the heating temperature could be described by a type of Arrhenius equation as follows¹⁴ in Eq. 4:

$$\ln k = \ln k_0 - \frac{E_a}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \quad (4)$$

Where:

- E_a = Activation energy (kJ mol⁻¹)
- k₀ = Thermal death rate at infinite temperature (sec⁻¹)
- R = Gas constant 8.314 J mol⁻¹ K
- T₁, T₂ = Absolute temperatures of thermal treatments (K)

The K, D, Z and E_a-values are essential parameters necessary to establish a continuous sterilization regime for molasses used in the baker's yeast industry. According to Ramaswamy *et al.*¹⁴, process time (F-value) for achieving a desired level of sterility could be calculated as follows in Eq. 5:

$$F_t = F_0 \cdot 10^{(T_0 - T)/Z} \quad (5)$$

Where:

- F₀ = Process time at reference temperature
- T₀ = A reference heating temperature (usually 121.1°C)
- T = Applied heating temperature

RESULTS AND DISCUSSION

Survivor curves of non-preheated spores of *G. stearothermophilus*. This part of experiment was carried out to explain the effect of industrial heat treatment of raw molasses on thermal death of *G. stearothermophilus* spores. The raw molasses was usually heated in baker's yeast company with steam injection and held at 90-95°C for 2 h, before it been diluted, cooled and pumped to fermentation tanks. Figure 1 show the survivor curves of *G. stearothermophilus* spores grown on TSB and molasses media heated at 90, 100, 110, 115 and 120°C (without preheating treatment of spore suspensions).

As seen, the survivor curves do not present a straight line on the semi-logarithmic scale. Most of the survivor curves show three phases of thermal inactivation, which confirm the observation of Iciek *et al.*⁵. Survivor curves obtained at 90, 100, 110 and 115°C starts with an initial fast death rate for the killing of vegetative cells, followed by two phases of very slow and slow death rates indicating simultaneous activation and

killing of *G. stearothermophilus* spores. However, heating at 120°C showed almost a straight line on semi-log scale indicating simultaneous activation and killing of the spores. At 90°C, the survivor curve flattened after 10 min, which indicate that this temperature is not sufficient to activate and kill the dormant and super dormant spores of *G. stearothermophilus*. From Fig. 1, it could be concluded that heating at 90°C is unsuitable treatment for killing spores of *G. stearothermophilus* but it could be applied for killing vegetative cells and for activation of spores of *G. stearothermophilus*. Abraham *et al.*¹³ applied heating at 93°C for 1 h, while Nolasco Junior and De Massaguer² applied heating at 98°C for up to 3 h to overcome the dormancy state of *B. stearothermophilus* spores. The biphasic destruction pattern of spores at 100, 110 and 115°C could be referred as described by Abraham *et al.*¹³, Iciek *et al.*⁵ and Nolasco Junior and De Massaguer² to the heterogeneity in spore thermal resistance and spore dormancy. The almost straight logarithmic line of thermal inactivation of spore populations at 120°C due, according to Iciek *et al.*⁵ to the shorter time

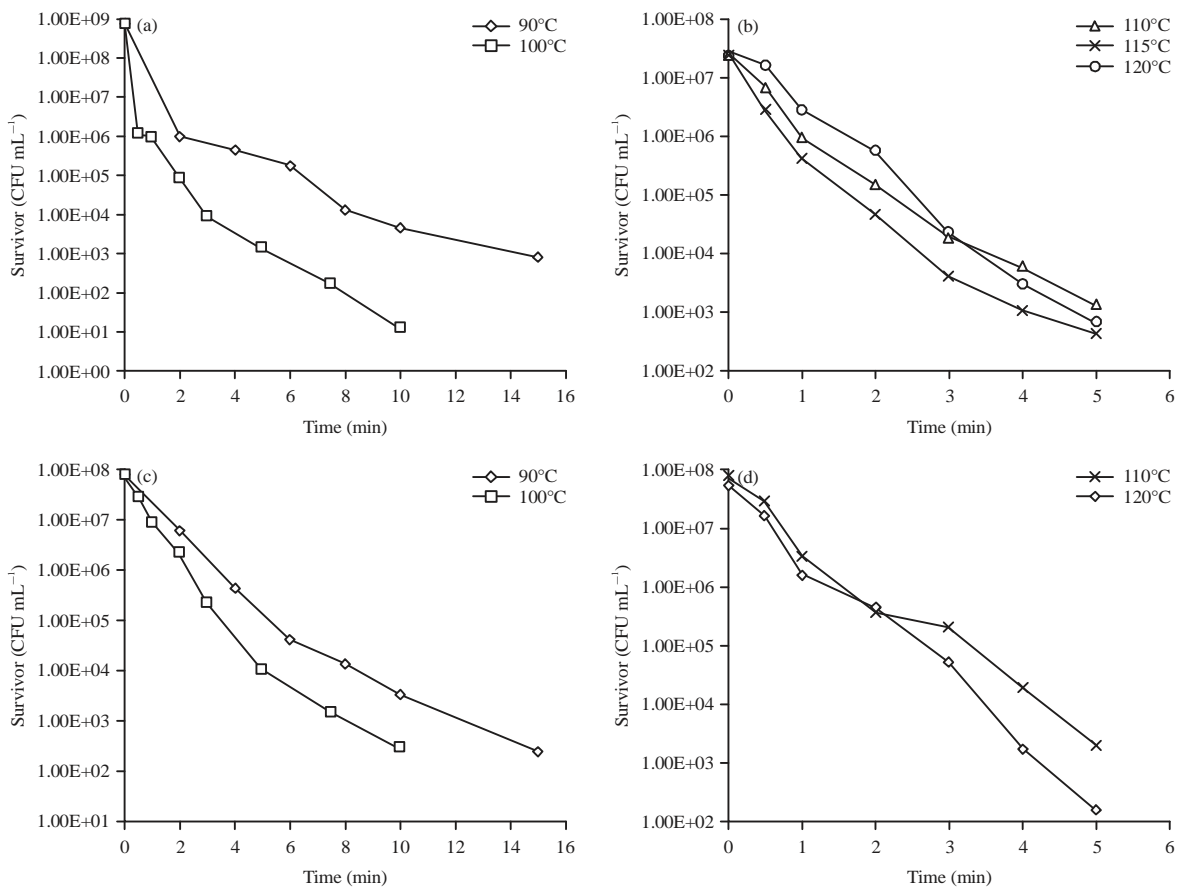


Fig. 1(a-d): Survivor curves of non-preheated *G. stearothermophilus* spores on (a, b) TSB and (c, d) Molasses media

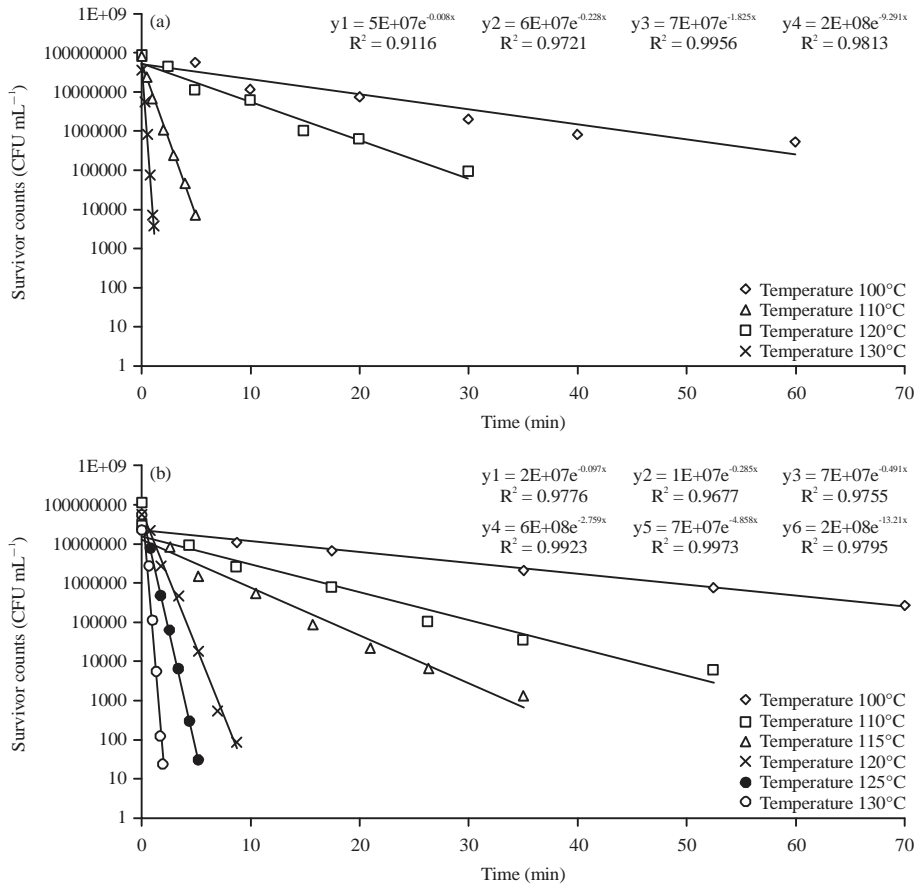


Fig. 2(a-b): Survivor curves of pre-heated *G. stearothermophilus* spores on (a) TSB and (b) Molasses media

required to reach a maximum number of activated spores and to the rapid rate of destruction of activated spores at such high temperature (120°C), where 90% inactivation of the spore population could be achieved at this temperature level within 6 min⁹.

Table 1 show the D-values of *G. stearothermophilus* calculated from trails of linearization of the survivor curves given in Fig. 1 on semi-logarithmic scale.

The D-values obtained at 90 and 100°C could not be considered due to the poor correlation coefficient (R²-values) for the treatments at these relatively low temperatures. At 110, 115 and 120°C, the R²-values are high (>0.95) and the D-values could be considered. However, the D-values obtained by using acidified molasses as heating media are lower than those obtained by TSB. These results agree with those of Abraham *et al.*¹³, who found that the logarithmic model was unable to describe the totality of the observed survival curve shapes of *B. stearothermophilus* spores at temperature <121.1°C. On other side, Iciek *et al.*^{5,15} stated that lowering the pH of the heating media resulted in accelerated killing rate of the heated spores.

Survivor curves of pre-heated spores suspensions of *G. stearothermophilus*

Figure 2 shows the survivor curves of *G. stearothermophilus* spores plotted on semi-logarithmic scale. The spores were pre-heated at 80°C for 10 min 1 h prior to thermal inactivation test. The spore suspensions were heated at temperature range of 100-130°C in TSB and in molasses media. As seen, the linear semi-logarithmic plot was obvious at temperatures 110, 120 and 130°C for spore suspensions heated in TSB media and at all temperatures (100, 110, 115, 120, 125 and 130°C) for those heated in molasses media. However, the survivor curves obtained at 100, 110 and 115°C in both media were characterized by the presence of an initial phase (4-10 min) of rapid inactivation followed by a linear phase of slower killing rate, while at higher temperatures (120 and 130°C) the survivor curves were mono-phasic linear on the semi-logarithmic plot during all the heating periods. It took 60 min heating time to induce a reduction of only two logarithmic cycles at 100°C, 30 min at 110°C for induction of three logarithmic cycles, while a reduction of 5-7 log cycles was achieved after 1.2 min heating at 130°C in both medium used. The initial required

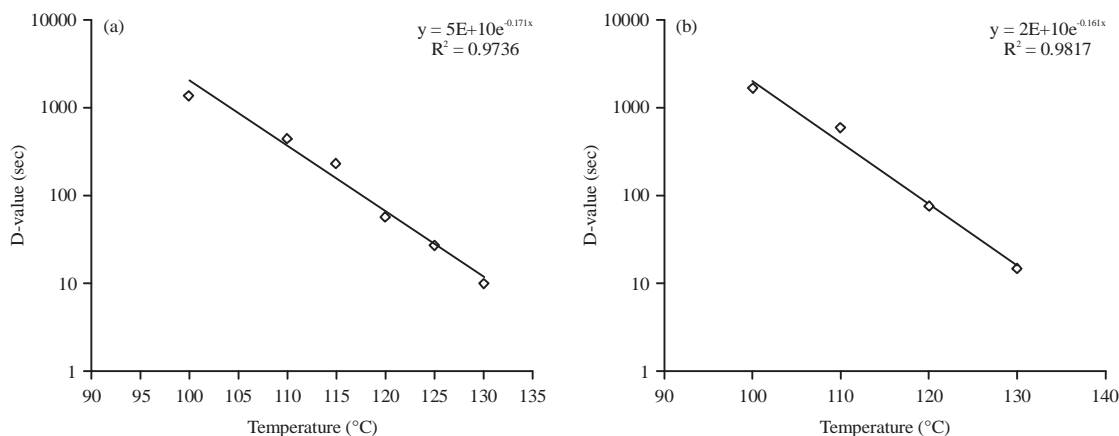


Fig. 3(a-b): Z-values *G. stearothermophilus* spores on (a) TSB and (b) Molasses

Table 1: Decimal reduction time of *G. stearothermophilus* suspensions at different temperatures (without preheating)

Temperature (°C)	Heated in TSB medium at pH = 7		Heated in molasses medium at pH = 4.7	
	D-value (min)	R ² -value	D-value (min)	R ² -values
90	9	0.847	7.5	0.955
100	4	0.827	3.8	0.985
110	2.5	0.975	2.2	0.976
115	1.80	0.953	1.72	0.981
120	0.98	0.987	0.93	0.988

inactivation phase at 100, 110 and 115 °C could be referred to the killing of vegetative cells present in the suspension.

However, at these relatively low temperatures, a killing tail was appeared at the end of the second killing phase, probably due to the presence of “super dormant” spores which could not be enough activated and killed at these relatively low temperatures.

Table 2 summarizes the thermal kinetics data for the killing of *G. stearothermophilus* spores in the used media (TSB and molasses media).

The reaction rate constant (K-values) as well as the decimal reduction time (D-values) present in Table 2 are higher than those obtained in Table 1 for spore suspensions tested without a pre-heating treatment, which indicates the importance of preheating treatment of crude spore suspensions to inactivate the vegetative cells and to activate the spores of *G. stearothermophilus* in order to make them more susceptible for thermal inactivation at the tested temperatures.

The K-values obtained with molasses media were higher than those obtained with TSB media, due to the lower pH (4.7) of the molasses, which support the thermal inactivation process. Accordingly, the D-values of *B. stearothermophilus* heated in molasses were lower than those obtained in the TSB media. Except of treatment at 100 °C in TSB media, the R²-values of the relationship between logarithm

of survival number and heating time were in the range of 0.972-0.997 indicating the suitability of the linear logarithmic modules (Eq. 1) to represent the survivor curves of *G. stearothermophilus* spores at temperature range of 110-130 °C.

The values of activation energy (E_a-values) for the killing process of *B. stearothermophilus* spores were calculated according to Eq. 4 and included in Table 2 and they were in the range of 200.163 and 212.22 kJ mol⁻¹, for spores heated in TSB and in molasses media, respectively. Higher E_a-values in molasses media reflect the effect of temperature increase on the acceleration of the spore killing process compared with TSB media. Figure 3 represents the Thermal Death Time (TDT)-curves of *G. stearothermophilus* killing under different temperatures in TSB and molasses media.

The TDT curves were plotted to determine the Z-value (Thermal death time constant), which is the temperature change (t) necessary to reduce the D-value by 90% (a measure for the variation of the thermal death rate with temperature). The obtained Z-values (Table 2) were 14.82 and 14.05 (°C) for the spores heated in TSB and molasses, respectively.

The presence of shoulders at the beginning of heating spore suspension or the presence of biphasic killing pattern was observed also by Yokoya and York¹², Feeherry *et al.*⁹, Abraham *et al.*¹³, Iciek *et al.*⁵, Nolasco Junior and De Massaguer² as well as Fraiha *et al.*¹⁶ at

Table 2: Thermal kinetics data for the killing of *G. stearothermophilus* spores in TSB and molasses media

Parameters	TSB media				Molasses media					
	100°C	110°C	120°C	130°C	100°C	110°C	115°C	120°C	125°C	130°C
K (min ⁻¹)	-0.088	-0.0228	-1.825	-9.291	-0.097	-0.285	-0.491	-2.759	-4.858	-13.21
D (min)	26.14	10.09	1.26	0.247	23.71	8.07	4.68	0.834	0.473	0.174
R ²	0.912	0.972	0.996	0.982	0.978	0.968	0.976	0.992	0.997	0.980
Z (°C)		14.82					14.05			
R ²		0.982					0.974			
K ₀ (min ⁻¹)		7.134 × 10 ²⁶					3.396 × 10 ²⁸			
E _a (kJ mol ⁻¹)		200.163					212.220			
R ²		0.977					0.978			

temperature range of 96-115°C for different strains of *B. stearothermophilus* spores. They referred this behavior to the inadequate pre-heating of the spore suspensions in order to kill vegetative cells and to activate dormant spores before the thermal killing experiments, as well as due to the variation in heat sensitivity within the same spore population.

The obtained values for K-(killing rate in min⁻¹) and D-values (Decimal reduction time in min) in the present study agree to a great extent with those found in the literature. The reported D-values for spore killing at 100°C were in the range of 29.4-72.9 min^{16,17}, while those reported for heating at 110°C were in the range of 8-53.4 min depending on the type of heating media and pH-value^{2,5,13,16}.

Reported D-values for *G. stearothermophilus* spores at 115°C were 2.8-40 min depending on strain, media type and pH-values^{5,9,13,15}. The obtained D-value for *G. stearothermophilus* in the present study at 100, 110 and 115°C (Table 2) either for heating in TSB or molasses media were lower than those reported in the literature. The reason for such difference could be referred to the strain of *G. stearothermophilus* used in the present study since there is a variation in heat resistance among the different strains of the microbe. On other side, the pre-heating condition (temperatures and time) were not the same among the studied research works. So, the percent of viable cells, activated spores and dormant spores differed in the populations used for thermal treatments at the temperatures range 100-115°C and hence the resulting survivor curves and their D-values varied also making this variation in the corresponding D-values.

However, D-values obtained in the present study at temperature range 120-130°C (Table 2) agree with those reported by Abraham *et al.*¹³, Nolasco Junior and De Massaguer², Iciek *et al.*¹⁵ and Fraiha *et al.*¹⁶. The reason for such agreement in the results could be referred to the completely linear course of the survivor curves of *G. stearothermophilus* spores on the semi-log scale at these

high temperatures, the instantaneous elimination of all sensitive fractions and the absence of dormant spores which could slow down the killing rate.

The values of activation energy E_a-values reported in the literatures^{2,17} varied between 193-249 kJ mol⁻¹, which agree with the E_a-values of the present study (200.16-212.22 kJ mol⁻¹). Also, the Z-values of 14.05 and 14.82°C given in Table 2, agree with the range of 10.13-16°C reported by Nolasco Junior and De Massaguer², Dogan *et al.*¹⁷ and Fraiha *et al.*¹⁶ for the specific thermal resistance (Z-values) of *G. stearothermophilus* spore suspensions.

Design parameters for molasses sterilization: Design of a proper sterilization treatment for molasses is based on the knowledge of spore thermal destruction discussed before. According to Ramaswamy *et al.*¹⁴ and Iciek *et al.*⁵, the spore destruction mechanism should be ruled by a first order kinetics over at least 5 logarithmic cycles to get appropriate parameters for the establishment of a thermal process.

Process time is based on achieving a desired level of sterility usually represented by a F_T-value corresponding to a selected reference temperature. According to Ramaswamy *et al.*¹⁴ and Iciek *et al.*¹⁵ different time temperature combination can be employed to achieve any desired level of sterility as follows in Eq. 6:

$$F_T = SV \times D_T \quad (6)$$

where, F_T is the treatment time (sec), D_T is the D-value at the treatment temperature (sec) and SV is the desired sterility level.

Process determination for continuous flow systems such as HTST involve the determination of the minimum holding tube length (L) required to inactivate a target microorganism in a given time (τ) at the process temperature¹⁸. Because there is a velocity distribution of the flow inside the holding tube, there is a resulting distribution of residence time of fluid in the

tube¹¹ which must be considered in calculating length (L) of the holding tube. For laminar flow ($Re < 2100$), as it is the case for flow of molasses in tubes, the layers flow through the center of the tube are faster than the average velocity of the liquid. Therefore in Eq. 7:

$$L = \tau \times v_{\max} \quad (7)$$

where, $\tau = F_T$ is a sterilization time, $v_{\max} = 2 v^-$ and v^- is an average flow velocity.

The HTST-system with direct steam injection provides conditions that promote efficient inactivation of spore with heat¹⁹. The molasses is raised instantaneously to the process temperature within less than 1 sec and held at this temperature during flow in the holding tube. According to Iciek *et al.*¹⁵ the inactivation time for *G. stearotherophilus* spores at a given temperature should be efficient to reduce the numbers of spores by 6-8 logarithmic cycles (Sterilization Value (SV) of 6-8). According to Nolasco Junior and De Massaguer², the continuous sterilization system for molasses leads to inactivation of contaminants with optimal sugar content retention. The sterilization time for molasses at temperatures higher than 130°C could be calculated using D-values estimated for microbial inactivation data (D and Z-values) obtained experimentally at temperatures lower than 130°C due to the difficulty of the very short sampling time needed at temperatures higher than 130°C to establish thermal death curves at these high temperatures⁸.

Based on the aforementioned information, a proposed flow design for the continuous sterilization of molasses under the conditions for baker's yeast production in Egyptian factories is given in Fig. 4. A production line in these factories

uses approximately 150-200 m³ of diluted (8° Brix) molasses per day for the production of baker's yeast. The production line starts with the transport of daily 20 t crude molasses (80%) to the clarifying tank and dilution with 20 t day⁻¹ of water to get a 40% molasses. The so diluted molasses (40 t day⁻¹) was clarified by acidification (phosphoric acid to neutralize the lime used in the manufacture of sugar and to lower the pH-value to 4.5-5), heated by direct steam injection to 95°C under mechanical agitation (2 h) and set for 3-4 h to collect the precipitate at the bottom of the clarifying tank. Due to the difficulty of filtration of cane molasses²⁰, clear molasses was syphoned and pumped at a rate approximately 3-4 m³ h⁻¹ for further processing. The advances of the clarifying step for the proposed sterilization process is the reduction of molasses viscosity from >50 Pa sec to 0.112 Pa sec due to dilution and heating from one side²¹ and the inhibition of vegetative microbial cells as well as activation of the dormant spores of *G. stearotherophilus* at the relatively high temperature (95°C) from other side. The hot clarified molasses (40%) was then pumped at a rate of 3.5 m³ h⁻¹ to a deaeration tank to expel the enclosed air under vacuum to avoid unnecessary pressure increase in the holding tubes thereafter. After deaeration, the 40% molasses is then pumped to a mixing chamber, where high pressure steam (3-5 bar) is directly mixed with the molasses to increase its temperature to 130-140°C level in less than 1 sec. The heated molasses flow through a holding tube for few seconds (at 130-140°C) to achieve the desired Sterilization Value (SV). A back pressure valve is mounted at the end section of the holding tube to allow only for the fully sterilized molasses to flow through, while under-sterilized molasses will be returned back to the steam mixing chamber. After sterilization, the hot molasses is

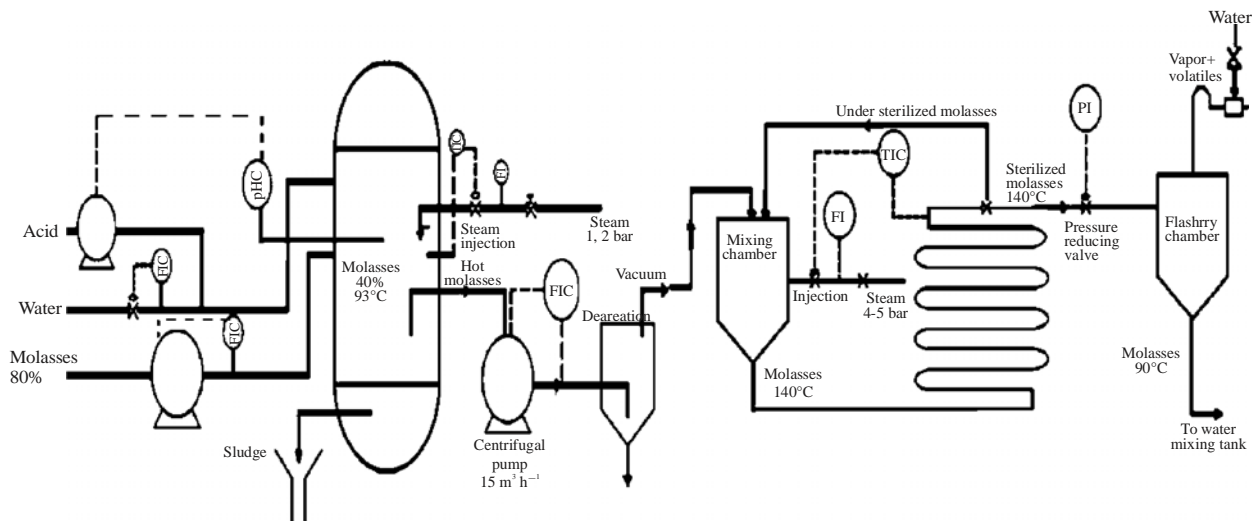


Fig. 4: Proposed flow design for the continuous sterilization of molasses under the conditions for baker's yeast production

then flash cooled in a vacuum chamber (flash vessel) to remove excess water as well as undesired volatile acids and odor from molasses, which leave the flashing chamber at 90-95°C. The proposed sterilization design agrees with the sterilization procedures described by Reed and Nagodawithana²². The so sterilized and flashed molasses is pumped to the water mixing tank in which it was mixed with water at rate of 4 kg water to each 1 kg sterilized 40% molasses to be diluted to 8% and pumped further to the fermentores at a temperature of ~30°C. The specific heat of 40% molasses is 0.802 kcal kg⁻¹ and 1 kg of 40% molasses at 93°C has a heat content (enthalpy) of 74.58 (kcal kg⁻¹) and upon mixing with 4 kg of water at 20°C (with enthalpy of 80 kcal) it will give a 5 kg diluted (8%) molasses with a temperature of 30.9°C.

Table 3 summarizes the design data for the holding tube at 3 different sterilization temperatures 130, 135 and 140°C.

The inside diameter of holding tube is chosen to be 5 cm (0.05 m), which guarantee a laminar flow pattern of molasses through the holding tube at a flow rate of 0.0012 m³ sec⁻¹ and an average velocity of 0.61 m sec⁻¹. The recommended flow velocity for 40% molasses in circular tubes should not exceed²⁰ 0.6-0.8 m sec⁻¹. Density and viscosity values of diluted molasses at 130, 135 and 140°C were obtained from data published by Tschubik and Maslow²³ as well as Olbrich²¹, who mentioned that the expansion ratio of molasses specific volume (m³ kg⁻¹) didn't exceed 0.043% in the range from

20-100°C and therefore, the change in the density of molasses by increasing temperature is negligible. The obtained Reynold numbers were in the range of 319-337 indicating that laminar flow pattern govern the flow of molasses during the continuous thermal sterilization and a velocity of 1.22 m sec⁻¹ equal to (2v⁻) should be taken in consideration for calculating the length of holding tube as seen in Table 3. The D-values for thermal death of *G. stearothermophilus* at 135 and 140°C were calculated based on the kinetic data given in Table 2. A Sterilization Value (SV) of 7 logarithmic cycles was considered in the present work according to recommendation published by Iciek *et al.*¹⁵ and Fraiha *et al.*¹⁶ for continuous sterilization of suspensions containing *G. stearothermophilus* spores.

The necessary sterilization time required to achieve the desired sterility ranged between 73.08 sec for heating at 130°C and 14.11 sec for heating at 140°C. The corresponding length of the holding tube is 89.16-17.21 m, respectively as seen in Table 3. Choosing 140°C as sterilization temperature will considerably reduce the holding tube length and the holding time. According to Wescolt *et al.*⁸ and Saravacos and Kostaropoulos²⁴, the temperature through the hold tubes decreases due to heat losses which may reduce the sterilization temperature and affecting the efficiency of the process. This heat losses are mainly due convection from the outside surface of the tube to the surrounding air, while the heat losses due to radiation could be neglected.

The heat losses through the surface of the holding tube were in the range of 17556 W (at 130°C and tube length of 89.16 m) to 3775.2 W (at 140°C and tube length of 17.21 m). In Table 3, the temperature drop due to the heat losses ranged between 4 and 0.89°C for heating at 130 and 140°C, respectively. This temperature drop can be compensated by heating molasses to 134, 137 and 141°C, respectively instead of 130, 135 and 140°C before entering the holding tube to avoid the need for thermal insulation of the holding tube.

The steam pressure required for mixing with the molasses ranged between 4 and 5 bar according to the temperature level required (Table 3), while the amount of steam could be set at 1 kg steam for each 10 kg molasses as recommended by Awuah *et al.*¹⁸. The sterilization times considered in the present study are slightly higher than the 58 sec at 130°C given by Quantz⁶ due to differences in molasses type from beet molasses to sugarcane molasses and the level of obtained sterility.

The pumping power required to move the molasses through the holding tube was calculated based on the friction losses through the holding tube and the pressure head

Table 3: Design data for the holding tube at 3 different sterilization temperatures

Design parameters	Sterilization temperatures (°C)		
	130	135	140
Flow rate of molasses (m ³ sec ⁻¹)	0.0012	0.0012	0.0012
Inside diameter of holding tube (cm)	5	5	5
Average flow velocity (m sec ⁻¹)	0.61	0.61	0.61
Density of molasses (kg m ⁻³)	1.170	1.160	1.150
Viscosity of molasses (mPa sec)	0.112	0.108	0.104
Reynolds number (Re)	319	328	337
Maximum velocity (m sec ⁻¹)	1.22	1.22	1.22
D-value (sec)	10.44	4.587	2.015
Sterilization value	7	7	7
Sterilization time (sec)	73.08	32.11	14.11
Length of holding tube (m)	89.16	39.17	17.21
Friction losses through tube length (m)	22.37	8.64	3.33
Head due to pressure (m)	36.15	41.82	45.01
Total head (m) (Friction+pressure)	58.52	50.46	48.34
Pump power by 65% efficiency (HP)	1.7	1.45	1.37
Steam pressure (bar)	4	4.5	5
Steam requirements for heating 10 kg molasses	1	1	1
Heat losses through the surface of holding tube (W)	17556	8147.5	3775.2
Temperature losses through the outer surface of holding tube (°C)	4	1.87	0.89
Corrected sterilization temperature (°C)	134	137	141

required to flow through the back pressure value and the data are included in Table 3. Friction losses were calculated according to Toledo²⁵ using friction factor (f), tube length (L), tube diameter (D) and flow velocity (v^{-}). The friction losses ranged between 22.37-3.33 m for sterilization at 130°C and 140°C, respectively. The pressure head in (m) varied according to the pressure of applied steam and it ranged from 36.15 m (at 130°C and absolute steam pressure of 3.5 bar) to 48.34 m (at 140°C and absolute steam pressure of 4.4 bar). Pressure losses due to velocity were neglected due to the low flow velocity of molasses through the holding tube. The required pumping power by 65% efficiency of the pump ranged between 1.7-1.37 HP.

CONCLUSION

By establishing such sterilization regime for molasses, the obtained baker's yeast will be free from contaminants of molasses origin (especially *Lactobacillus* and *Bacillus* species), which will increase both yield and performance of baker's yeast.

REFERENCES

1. Pillai, J.S., N. Danesh, E.T. Puttaiah and K. Girish, 2011. Microbial diversity in solid waste molasses of sugar industry, Aranthangi, Tamilnadu. Int. J. Environ. Sci., 2: 723-730.
2. Nolasco Junior, J. and P.R. De Massaguer, 2007. Thermal death kinetics of *B. stearothermophilus* spores in sugarcane must. J. Food Process Eng., 30: 625-639.
3. Salem, S.H., M.M. Naguib, H.H. El-Sheikh and Y.A. Heikal, 2015. Determination of the microbial profile of compressed baker's yeast and studying its symbiotic interactions. Int. J. Adv. Res., 3: 303-312.
4. Kristiansen, B., 1994. Integrated Design of a Fermentation Plant: The Production of Baker's Yeast. VCH Publishing, Germany, ISBN: 9781560817666, pp: 53-56.
5. Iciek, J., A. Papiewska and M. Molska, 2006. Inactivation of *Bacillus stearothermophilus* spores during thermal processing. J. Food Eng., 77: 406-410.
6. Quantz, M., 2015. Molasses Treatment. VH Lecture, Lectures published by research institute for Baker's yeast (VH-Berlin), Germany.
7. Stumbo, C.R., 1973. Thermobacteriology in Food Processing. 2nd Edn., Academic Press, New York.
8. Wescolt, G.G., T.M. Fairchild and P.M. Foegeding, 1995. *Bacillus cereus* and *Bacillus stearothermophilus* spore inactivation in batch and continuous flow systems. J. Food Sci., 60: 446-450.
9. Feeherry, F.E., D.T. Munsey and D.B. Rowley, 1987. Thermal inactivation and injury of *Bacillus stearothermophilus* spores. Applied Environ. Microbiol., 53: 365-370.
10. Dlugokenski, R.E., S.R. Sella, B.P. Guizelini, L.P. Vandenberghe, A.L. Woiciechowski, C.R. Soccol and J.C. Minozzo, 2011. Use of soybean vinasses as a germinant medium for a *Geobacillus stearothermophilus* ATCC 7953 sterilization biological indicator. Applied Microbiol. Biotechnol., 90: 713-719.
11. Wang, D.I., J. Scharer and A.E. Humphrey, 1964. Kinetics of death of bacterial spores at elevated temperatures. Applied Microbiol., 12: 451-454.
12. Yokoya, F. and G.K. York, 1965. Effect of several environmental conditions on the thermal death rate of endospores of aerobic, thermophilic bacteria. Applied Microbiol., 13: 993-999.
13. Abraham, G., E. Debray, Y. Candau and G. Piar, 1990. Mathematical model of thermal destruction of *Bacillus stearothermophilus* spores. Applied Environ. Microbiol., 56: 3073-3080.
14. Ramaswamy, H.S., F.V.D. Voort and S. Ghazala, 1989. An analysis of TDT and Arrhenius methods for handling process and kinetic data. J. Food Sci., 54: 1322-1326.
15. Iciek, J., I. Blaszczyk and A. Papiewska, 2008. The effect of organic acid type on thermal inactivation of *Geobacillus stearothermophilus* spores. J. Food Eng., 87: 16-20.
16. Fraiha, M., A.C.D.O. Ferraz and J.D. Biagi, 2010. Determination of thermobacteriological parameters and size of *Bacillus stearothermophilus* ATCC 7953 spores. Cienc. Tecnol. Aliment., 30: 1041-1045.
17. Dogan, Z., K. Weidendorfer, M. Muller-Merbach, F. Lembke and J. Hinrichs, 2009. Inactivation kinetics of *Bacillus* spores in batch-and continuous-heating systems. LWT-Food Sci. Technol., 42: 81-86.
18. Awuah, G.B., H.S. Ramaswamy and A. Economides, 2007. Thermal processing and quality: Principles and overview. Chem. Eng. Process.: Process Intensification, 46: 584-602.
19. Busta, F.F., 1967. Thermal inactivation characteristics of bacterial spores at ultrahigh temperatures. Applied Microbiol., 15: 640-645.
20. Anonymous, 1980. The Production of Sugar. Scientific Publishing, Leipzig, Germany, pp: 377-378.
21. Olbrich, H., 2006. The Molasses. In: Principles of Sugar Technology, Olbrich, H. (Ed.). Biotechnologie-Kempe GmbH Publishing, London, UK., pp: 131-144.
22. Reed, G. and T.W. Nagodawithana, 1991. Yeast Technology. 2nd Edn., Van Nostrand Reinhold, New York.
23. Tschubik, I.A. and A.M. Maslow, 1973. Thermophysical Properties of Foodstuffs and Semi Products. VEB Fachbuchverlag, Leipzig.
24. Saravacos, G.D. and A.E. Kostaropoulos, 2002. Handbook of Food Processing Equipment. Kluwer Academic/Plenum Publisher, New York, ISBN: 9780306472763, Pages: 698.
25. Toledo, R.T., 1997. Fundamentals of Food Process Engineering. 2nd Edn., CBS Publishers and Distributors, New Delhi, pp: 209-219.