# HEAT TREATMENT OF NON-CONCENTRATED TOMATO PRODUCTS

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Industrial sterilization processes for preserved foods have the purpose of ensuring microbiological and enzymatic stabilization of the products, to enable preservation at room temperature and, therefore, deferred consumption.
The microbiological stability of preserved foods is influenced by the effectiveness of the heat treatments used, by the tightness of containers and by storage temperatures. The spoilage of a preserved food is thus the consequence of an ineffective heat treatment, insufficient tightness of the containers or unsuitable cooling of the packs and/or storage at high temperatures.
In the first case, a fraction of the microbial population will survive and develop in the product; in the second case, the microorganisms present in the external environment (cooling water, air, surfaces, etc.) will penetrate in containers and pollute food. The permanence of containers at high temperatures can cause the growth of the thermophilic bacteria that survived the heat treatment *(1-4)*.
The microbial stabilisation of a sterilised product is assessed in terms of probability of survival of the microorganisms present before the heat treatment. Indeed, it is not possible to achieve absolute sterility in a microbial population through heat treatments because the inactivation of microorganisms with heat follows an exponential law *(1, 2, 5- 8)*.
For these reasons the concept of "commercial sterility" has been introduced and it refers to the sterility obtained when the probability of survival of the microorganisms that are capable of growing inside the product concerned and thus the possibility of spoilage of the food preserve is reduced to levels considered acceptable.
The magnitude of the heat treatment required to stabilize a preserved food microbiologically can be defined when the following elements are known:
1) the heat resistance ($D_T$ and z) in the same substrate of the most heat resistant microorganism that may contaminate it and that can grow
2) the real or supposed concentration of cells (or spores) of such microorganism.
Furthermore, the acceptable probability of survival should be defined in terms of "probability of a non-sterile unit" *(PNSU)*, also as the determination of the endpoint of the industrial heat sterilization process *(6, 9)*.
The "sterilizing effect" or "lethal effect" $F_T^z$ is the time required to obtain a defined microbial inactivation at a given temperature *(2, 5-8)*.
The resulting heat treatment must therefore be such to guarantee the achievement of commercial sterility of the product concerned.
The sterilizing effect or lethal effect can be calculated using the following formula *(10, 11)*:

$$F_T^z = D_T \times (\text{Log } N_0 - \text{Log } N_F) = D_T \times n$$

where:

- $D_T$ = time for decimal reduction of the reference microorganism to be inactivated (time required to inactivate, at a constant temperature T, 90% of the initial microbial cells or spores);
- z = range of variation of the maintenance temperature T, for which the value $D_T$ increases or diminishes by 10 times;
- $N_0$ = measured or estimated initial microbial concentration;
- $N_F$ = probability of survival considered acceptable *(PNSU)*;
- n = number of resulting decimal reductions.

The reference microorganism therefore needs to be identified to calculate the sterilizing or lethal effect to be applied to a food.

For this reason, preserved foods are subdivided according to the pH values into "acid" foods with pH ≤ 4.60 and "non-acid" foods with pH > 4.60 according to the different development capacity and heat resistance of microorganisms based on this parameter *(1, 7, 12, 13)*.
These two groups then undergo heat treatments of different magnitude.
For preserved foods having pH ≤ 4.60 the heat treatment is generally performed at temperatures not exceeding 100°C; this has the purpose of inactivating the spores (as well as the cells) of the spoilage microorganisms, which are therefore able to grow at low pH values, but not the spores of microorganisms that are not able to germinate in these acid environments. Preserved foods with pH > 4.60 undergo a more intense sterilization treatment that has the purpose of inactivating all the spores present in the product since they are potentially able to grow in it since the pH does not have an inhibiting function for spore germination; all the vegetative cells will be thus inactivated because of their lower heat resistance *(5- 7, 13, 14)*.
The use of different heat treatments is essentially due to the different possibility of development, based on the pH value, of the pathogenic bacterium *Clostridium botulinum*, which is a microorganism of huge health relevance since it causes lethal intoxication due to the ingestion of neurotoxins produced after the development of the bacterium in food. Scientific data shows that the minimum pH inhibiting the development (germination) of *C.botulinum* spores is 4.60 *(15- 18)*.
Another parameter influencing the magnitude of heat treatments is the value of $a_w$ *(*activity of water*)*; below 0.90 generally no spore germination occurs *(19- 21)*.
The sterilizing effect $\boldsymbol{F_T^z}$ is usually calculated at reference temperatures T of 85 or 95°C for preserved foods *(including very acidified tomato products)* with pH < 4.2 and tomato paste, of 100°C for preserved foods having 4.2 ≤ pH ≤ 4.6 and at a temperature of 121°C for preserved foods with pH > 4.6 *(22)*.
In non-concentrated tomato preserves (4.2 ≤ pH ≤ 4.6) some microorganisms with poor heat resistance can grow, such as lactic bacteria, enterobacteria, yeasts and moulds and some species of sporogenic bacteria such as "butyric clostridia" (*Clostridium pasteurianum, C. butyricum, C. acetobutylicum,* etc.), *Bacillus coagulans* and, more rarely, *Paenibacillus macerans*, *Paenibacillus polymyxa, Thermoanaerobacterium thermosaccharolyticum* and *Alicyclobacillus acidocaldarius (3, 4, 13, 14, 22-30)*.
Vegetative cells in most cases are inactivated rapidly already at temperatures around 60-65°C: the values of $D_{65\text{-}60}$ are close to 1-5 minutes *(5, 7)*.
The mesophilic *Bacillus* and *Clostridium* species that can develop in acid products, even forming spores with lower heat resistance values compared to other species of the same genus, are however resistant to treatments with temperatures close to 100°C *(2, 3, 7, 13, 14, 23, 24)*.
The stabilization of non-concentrated acid products can be achieved only inactivating or inhibiting the spores of such bacteria.
Non-concentrated tomato products, and in particular peeled tomatoes, are frequently subject to spoilage by butyric clostridia that shows itself with an evident swelling of containers, reduction of pH, production of butyric acid and development of off-odours.
The decimal reduction time of the spores of *C. pasteurianum* (the most heat resistant amongst butyric clostridia) in tomato juice at pH = 4.5 *(with count of the surviving spores in culture medium with the same pH)* is 0.65 minutes at 100°C, with z = 10°C *(31)*.
The maximum contamination found in fresh tomato is about $10^4$ spores per gram *(32)*.
The thermal stabilization of peeled tomatoes and similar products thus requires the application of times and temperatures taking into account the maximum contamination of the fresh product and the final probability of spoilage considered acceptable.
In industrial practices, with pH values of 4.5, accepting a spoilage probability $N_F$ of 1 out of $10^5$ containers ($10^{-5}$) weighing 400 g the result will be:
$\mathbf{F_{100}^{10}}$ = 0.65' x [log ($10^4$ x 4 ·$10^2$) – log $10^{-5}$] = 0.65' x [log (4 ·$10^6$) – log$10^{-5}$] = 0.65' x 11.6 = 7.5'.
All in all, a high microbiological stability/commercial sterility is obtained with heat treatments of which the lethal effect is:
$\mathbf{F_{100}^{10}}$ ≥ 7.5 minutes.
Or, in the case of containers weighing 800 g:
$\mathbf{F_{100}^{10}}$ = 0.65' x [log ($10^4$ x 8 ·$10^2$) – log $10^{-5}$] = 0.65' x [log (8·$10^6$) – log$10^{-5}$] = 0.65' x 11.9 = 7.7'.
All in all, a high microbiological stability/commercial sterility is obtained with heat treatments of which

the lethal effect is:
$F_{100}^{10} \geq 7.7$ minutes.

In the calculation made, the maximum value of contamination from butyric clostridium spores considered is to be deemed high since it can be found in unsuitable transport and holding conditions of the fresh tomato supplied; the improvement of harvesting and transportation systems can diminish the maximum level of microbiological pollution and, therefore, the magnitude of the sterilizing effect can be diminished.
The possibility of spore germination and thus of the onset of spoilage also depends on the finished equilibrium pH values; the probability of development of spores indeed diminishes with the reduction of the pH value. Literature shows a reduction of the probability of spore germination by 100 times with every reduction of pH by 0.1 units of packaged product *(33)*; using a precautionary principle, one can consider, more cautiously, a reduction of the probability of 10 times with every reduction of pH by 0.1 units.
It is therefore possible to diminish the magnitude of the lethal effect according to the finished equilibrium pH value.
For example, bringing the product pH value to 4.30 in an accurate and homogeneous way means reducing the initial population by 2 orders of magnitude; these "*equivalent*" decimal reductions can be added to the ones resulting from the heat treatment.
The formula given below is the one used for calculating the sterilizing effect for a tomato product with pH = 4.30 where, taking into account the low probability of germination at such pH, the degree of spore pollution considered is $10^2$/g instead of $10^4$/g.
For packages weighing 400 g:
$F_{100}^{10}$ = 0.65'x [log ($10^2$ x 4 ·$10^2$) – log $10^{-5}$] = 0.65' x [log (4 ·$10^4$) – log$10^{-5}$] = 0.65' x 9.6 = 6.2 minutes.
For packages weighing 800 g:
$F_{100}^{10}$ = 0.65'x [log ($10^2$ x 8 ·$10^2$) – log $10^{-5}$] = 0.65' x [log (8 ·$10^4$) – log$10^{-5}$] = 0.65' x 9.9 = 6.4 minutes.

Of the species mentioned above, the most heat resistant bacterium amongst the ones that can spoil non-concentrated tomato products because of an insufficient heat treatment is *B. coagulans (facultative thermophilic-mesophilic)*, causing flat-sour, spoilage with reduction of pH, but no or very limited development of gas and development of off-odours *(medicinal – phenolic) (1, 19, 26, 30, 34-38)*.
The levels of contamination for fresh tomato show a high variability (from 0 to 1.8 •$10^3$ spores/g), but 5.5 • $10^2$ spores/g *(39)* can be assumed as the most probable maximum level (p = 0.95).
With regards to the heat resistance of spores of *B. coagulans*, it was recently measured on six strains that are responsible for the spoilage of tomato preserves by flat-sour, in tomato juice at pH values of 4.3 and 4.5 and count of surviving spores in culture medium with the same pH *(40)*.
At pH = 4.5 the highest decimal reduction time at 100°C was 0.79 minutes ($D_{100}$ = 0.79'); at pH 4.3 the maximum value of $D_{100}$ was 0.58 minutes ($D_{100}$ = 0.58').
At pH = 4.3, for *B. coagulans*, a value of $F_{100}$ = 6.0 minutes is calculated applying to the 400 g pack the formula $F_T^z = D_T \times (\text{Log } N_0 - \text{Log } N_F)$ with the maximum value of $D_{100}$ found and with a final probability of maximum spoilage by *B. coagulans (PNSU probability of a non-sterile unit*) of 1 pack out of $10^5$ i.e. $10^{-5}$.
At a pH of 4.3 the value $F_{100}$= 6.2 minutes calculated previously for *C. pasteurianum* is therefore sufficient also for *B. coagulans* with *PNSU* of $10^{-5}$.
At pH = 4.5 instead, for *B. coagulans*, a value of $F_{100}$ = 8.2 minutes is calculated with *PNSU* of $10^{-5}$; this value of the necessary sterilizing effect is slightly higher than the one calculated for *C. pasteurianum* with the same final probability of spoilage.
At a pH of 4.5 the value $F_{100}$ = 7.5 minutes calculated for *C. pasteurianum* in tomato products is effective also for *B. coagulans*, accepting $10^{-4}$ as *PNSU* for the latter.
Similar considerations apply to the 800g pack with both pH values.
However, one should consider that spoilage by *B. coagulans* in tomato products undergoing the usual heat treatments at temperatures close to 100°C is not frequent; it refers almost exclusively to heat treated products at temperatures exceeding 110°C: it is possible that a considerable increase of the z value of spores occurs in these conditions.
In these cases the recommended lethal effect of the heat treatment is the following: $F_{121}$ = 0.7 minutes

*(34)*.
It must be noted that in previous research carried out on the effect of sub-lethal heat treatments having as an effect only two decimal reductions on $10^4$ spores/ml of strains of *B. coagulans*, isolated from tomato products spoiled for flat-sour, heat treatments proved to be suitable to prevent spore growth in tomato juice at pH 4.45. The surviving spores, amounting to $10^2$/ml, quickly inactivate (about 30 days) thanks to the synergistic action of acidity, hydrogen-ion concentration and sub-lethal heat treatment *(22, 35, 41)*.
The table below shows the values of $F_{100}$ with the two pH values and the respective final probabilities of spoilage (*PNSU*) for the two sporogenic microorganisms concerned.

***Values of the sterilizing effect $F_{100}$ and probability of spoilage***

| Value of pH* (*finished equilibrium*) | $F_{100}$ | PNSU – *C. pasteurianum* | PNSU – *B. coagulans* |
|---|---|---|---|
| 4.3 *(400g)* | 6.2 minutes | $10^{-5}$ | $10^{-5}$ |
| 4.3 *(800g)* | 6.4 minutes | $10^{-5}$ | $10^{-5}$ |
| 4.5 *(400g)* | 7.5 minutes | $10^{-5}$ | $10^{-4}$ |
| 4.5 *(800g)* | 7.7 minutes | $10^{-5}$ | $10^{-4}$ |

** Small oscillations (e.g.: 0.05 units pH) around the values are irrelevant in industrial practices.*

With regards to sporogenic thermophilic bacteria, they (*as spores*) have a high resistance to heat; the magnitude of heat treatments cannot be adapted to their values of $D_{100}$ since they would be so intense to cause a degradation of the organoleptic features of the products.
In non-concentrated tomato products the spoilage by *Thermoanaerobacterium thermosaccharolyticum* (anaerobic, gasogenous) and by *Alicyclobacillus acidocaldarius*, in anomalous aerobiosis conditions, is relatively frequent.
They cause spoilage only when the holding temperatures of juices, sauces, chopped products, etc. in the tanks along the processing line or at the end of cooling and/or storage of packs, are high enough to enable their development.
The spores of *Thermoanaerobacterium thermosaccharolyticum* are present in the raw material at maximum concentrations of $3 \cdot 10^3$/g in filling juices and $4.5 \cdot 10^2$/g in peeled tomatoes *(42)*. Such spores have very high resistance to heat; the value of $D_{115}$ at pH 4.5 varies from 3.3 to 8.7 minutes depending on the various strains tested with z values from 7.3 to 8.3°C, and with pH 4.3 it is 3.8 – 6.3 minutes and z from 7.8 to 9.3°C *(43)*.
The spores of *Alicyclobacillus acidocaldarius* are frequently found in tomato products, but at very low concentrations, i.e. few units per gram of product.
These spores have a fairly high heat resistance: $D_{100}$ of 18.9 – 21.7 minutes with z values of 6.45 and 11.12°C, respectively *(44)*.
These microorganisms grow only at temperatures of 37-65°C and in the presence of oxygen at concentrations higher than the ones present in preserved food packs *(45, 46)*.
Spoilage is thus more likely along the processing line, especially with holding times in tanks at temperatures suitable for growth; this causes a decay of the organoleptic features of the product (*off-flavour*) *(46)*.
With the purpose of reducing the risk of spoilage by these microorganisms, a good quality of the raw material, an appropriate washing (*Alicyclobacillus spp.* comes from soil), good hygiene conditions in processing, a rapid and effective cooling after the heat treatment, the reduction of holding times in tanks and the storage of the packaged products at temperatures below 37°C are fundamental to prevent the possible germination of surviving spores.
Based on the data available, the heat treatments contained in the table are deemed to provide an adequate level of commercial sterility with regards to the possibility of spoilage by *C.pasteurianum* and by *B.coagulans* in products stabilized using the usual heat treatments carried out at temperatures close to 100°C.
Reductions of the magnitude of the sterilizing effect can be carried out by diminishing the pH value

of products in an accurate and homogeneous way and/or reducing the initial concentration of the spores of spoilage bacteria through an improved washing of raw materials and general hygiene of processing lines.
The spore concentration in raw materials can be determined analytically and included in the formula to calculate the sterilizing effect.

***This report was written using the data collected during the research activities of the Microbiology Department of SSICA.***
***It updates and completes the previous version:***
***"Thermal stabilization of non-concentrated tomato products", G.Pirone, M. P.Previdi, G. Dipollina, Ind. Conserve, 87, 141 (2012).***

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