ECOLOGIA MICROBIANA DE ALIMENTOS

- CARLOS DIAS PEREIRA

- ESAC

- INFORMAÇÃO ADAPTADA DE ICMSF (1980)

Intrinsic And Extrinsic Parameters Influencing Microbial Growth In Foods

Intrinsic factors	Extrinsic factors
pH	storage temperature
a_w	relative humidity of environment
E_h	presence and concentration of gases in the environment
nutrient content	
antimicrobial constituents	
biological structures	

Classification Of Undesirable Changes That Can Occur In Foods

Attribute	Undesirable change
Texture	a. Loss of solubility
	b. Loss of water-holding capacity
	c. Toughening
	d. Softening
Flavor	Development of
	e. Rancidity (hydrolytic or oxidative)
	f. Cooked or caramel flavors
	g. Other off-flavors
Color	h. Darkening
	i. Bleaching
	j. Development of other off-colors
Appearance	k. Increase in particle size
	l. Decrease in particle size
	m. Non-uniformity of particle size
Nutritive value	Loss or degradation of
	n. Vitamins
	o. Minerals
	p. Proteins
	q. Lipids

Chemical Reactions That Can Lead To Deterioration Of Food Quality Or Impairment Of Safety

- Nonenzymic browning
- Lipid hydrolysis
- Lipid oxidation
- Protein denaturation
- Protein cross-linking
- Oligo- and polysaccharide hydrolysis
- Protein hydrolysis
- Polysaccharide synthesis
- Degradation of specific natural pigments
- Glycolytic changes

Table1. Range of temperatures (ºC) of growth of the four major physiological groups of bacteria.

GROUPS	Minimum	Optimum	Maximum
THERMOPHILES	40	55-75	90
MESOPHILES	5	30-45	47
PSYCHROTROPHS	-5	25-30	35
PSYCHROPHILES	-5	12-15	20

Adapted from ICMSF (1980).

Table 2. Decimal reduction times (D values) of some microorganisms at specific temperatures.

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MICROORGANISMS	Temperature (ºC)	D value (minutes)
Brucella spp **ϕ**	**65,5**	**0,1-0,2**
Salmonella senftenberg 775W **ϕ**	**65,5**	**0,8-1,0**
Salmonella **spp. ϕ**	**65,5**	**0,02-0,25**
Mycobacterium tuberculosis **ϕ**	**65,5**	**0,20-0,30**
Coxiella burnetii **ϕ**	**65,5**	**0,50-0,60**
Listeria monocytogenes **ϕ**	**71,7**	**0,03-0,6**
Staphylococcus aureus **ϕ**	**65,5**	**0,2-2,0**
Yeasts; Molds; Spoilage microorganisms	**65,5**	**0,5-3,0**
Lactococcus lactis	**65**	**0,01**
Escherichia coli **ϕ***	**65**	**0,10**

ϕ: microorganisms that represent food hazards.
ϕ* Five pathogenic groups of *E. coli* are nowadays identified (Jay,1999).
Adapted from ICMSF(1980).

Table 3. Decimal reduction times (D values) of some spores at specific temperatures.

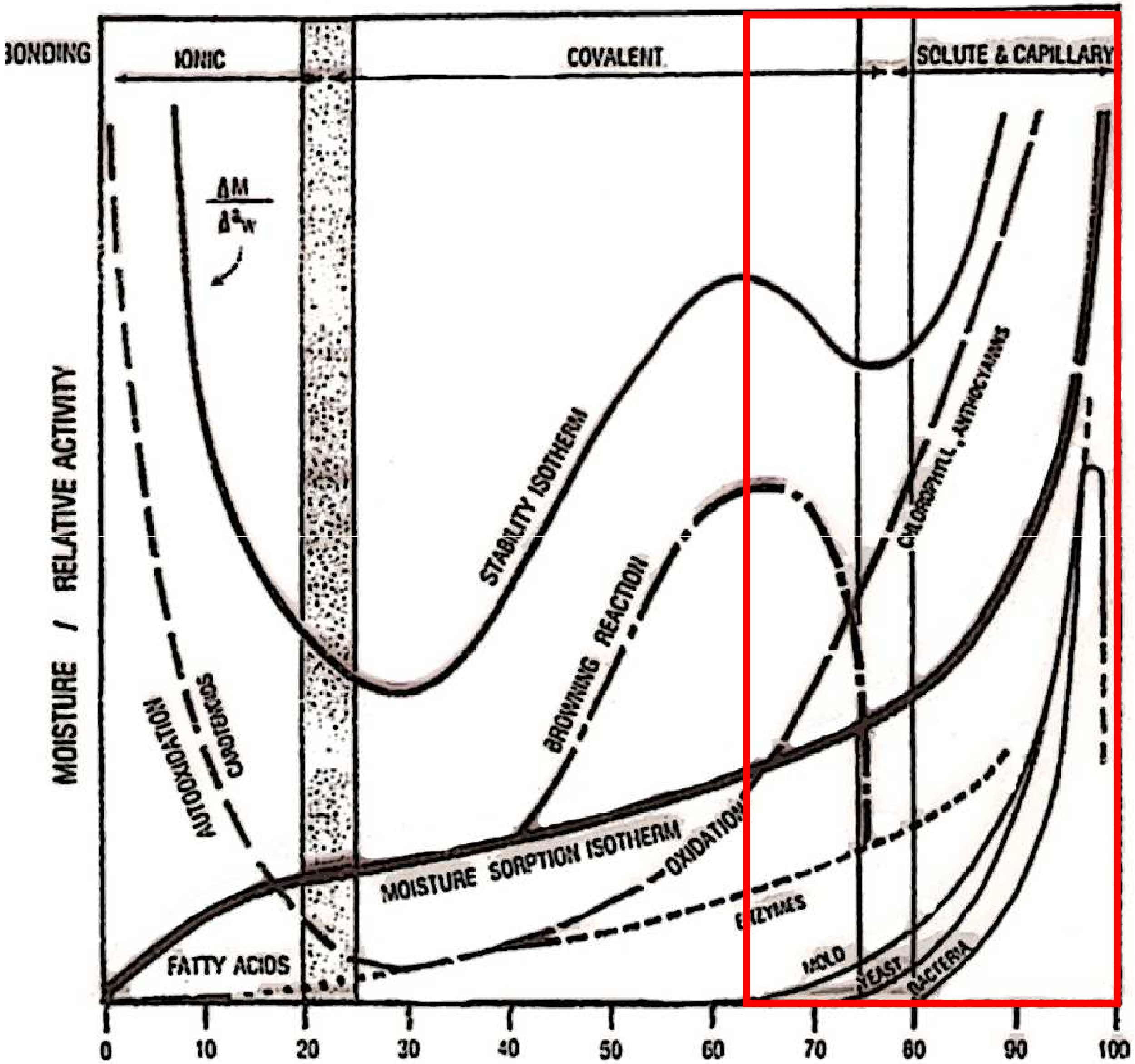
MICRORGANISMS	Temperature (ºC)	D value (minutes)
mesofílic aerobe spores		
Bacillus cereus ϕ	100	5,00
Bacillus subtilis	100	11,00
Bacillus polymyxa	100	0,1-0,5
mesofílic anerobe spores		
Clostridium butyricum	100	0,1-0,5
Clostridium perfringens ϕ	100	0,3-20
Clostridium botulinum ϕ	100	
A and B: proteolitic	100	50,00
E and non proteolitic B and F	80	1,00
termofílic aerobe spores		
Bacillus coagulans	120	0,10
Bacillus stearothermophilus	120	4,0-5,0
termofílic anerobe spores		
Clostridium thermosaccharolyticum	120	3,0-4,0
Clostridium nigrificans	120	2,0-3,0

ϕ: microorganisms that represent food hazards
Adapted from ICMSF (1980).

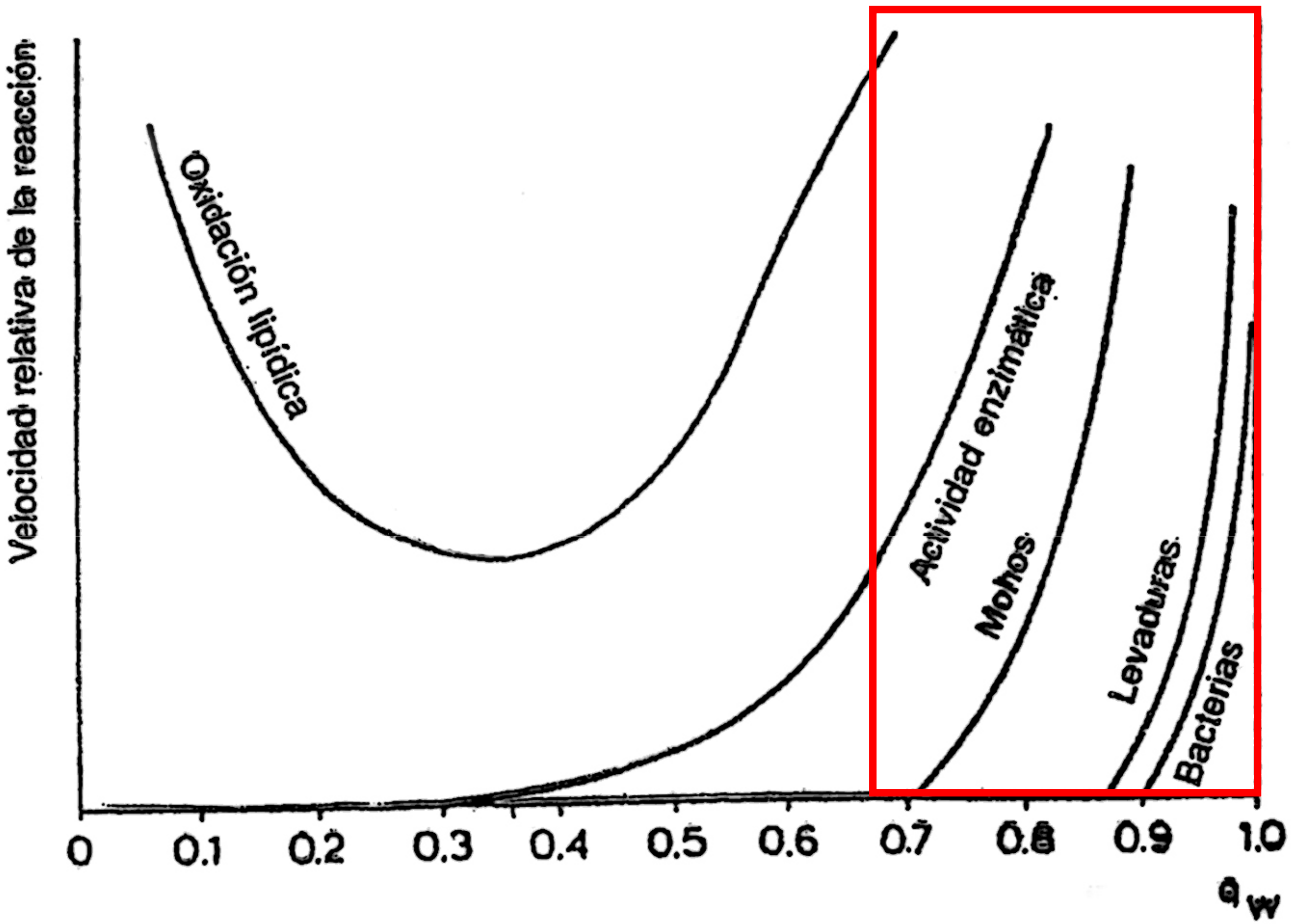
Table 4. Minimum levels of water activity allowing growth of microorganisms at temperatures near optimal.

MICROORGANISM	Minimum a_w
BACTERIA	
Clostridium botulinum type E ϕ	0,97
Clostridium botulinum type B ϕ	0,94
Clostridium botulinum type A ϕ	0,95
Bacillus cereus ϕ	0,95
Clostridium perfringens ϕ	0,95
Escherichia coli ϕ	0,95
Salmonella spp ϕ	0,95
Staphylococcus aureus ϕ	0,86
YEASTS	
Debaryomyces hansenii	0,83
Saccharomyces bailii	0,80
Saccharomyces cerevisiae	0,90
MOLDS	
Alternaria citri	0,84
Aspergillus níger ϕ	0,77
Aspergillus flavus ϕ	0,78
Botrytis cinerea	0,93
Rhizopus nigricans	0,93
Penicillium chrysogenum	0,79

ϕ: microorganisms that represent food hazards
Adapted from ICMSF, (1980).



BONDING
IONIC
COVALENT
SOLUTE & CAPILLARY
MOISTURE / RELATIVE ACTIVITY
ΔM
Δ²w
STABILITY ISOTHERM
BROWNING REACTION
CHLOROPHYLL, ANTHOCYANINS
AUTOOXIDATION
CAROTENOIDS
MOISTURE SORPTION ISOTHERM
OXIDATION
ENZYMES
FATTY ACIDS
MOLD
YEAST
BACTERIA
0
10
20
30
40
50
60
70
80
90
100



Reacciones químicas y biológicas frente a la actividad de agua.

Approximate Minimum a_w Values For The Growth Of Microorganisms Of Importance In Foods

Organism	Minimum a_w
Most spoilage bacteria	0.91
Most spoilage yeasts	0.88
Most spoilage molds	0.80
Halophilic bacteria	0.75
Xerophilic molds	0.65
Osmophilic yeasts	0.60

Table 5. Approximate levels of water activity of five groups of food commodities.

FOOD COMMODITIES	WATER ACTIVITY
	1,0-0,98
FRESH MEAT/FISH	
MILK AND BEVERAGES	
YOGHURT/SOFT CHEESES	
FRESH FRUITS AND VEGETABLES	
	0,98-0,93
LIGHTLY SALTED BEEF PRODUCTS	
FERMENTED SAUSAGES	
EVAPORATED MILK	
CANNED CURED MEATS	
SEMI HARD CHEESE	
	0,93-0,85
DRIED MEAT/FISH	
SEMI SOFT/HARD; HARD CHEESES	
CONDENSED MILK	
RAW CURED HAM	
	0,85-0,60
DRIED FRUIT	
FLOUR	
CEREALS	
JAMS AND JELLIES	
SOME HARD CHEESES	
HEAVILY SALTED FISH	
NUTS	
	<0,60
HONEY	
BISCUITS	
DRIED MILK	

Adapted from ICMSF (1980).

Table 7. Lower limits o pH allowing growth of various microorganisms.

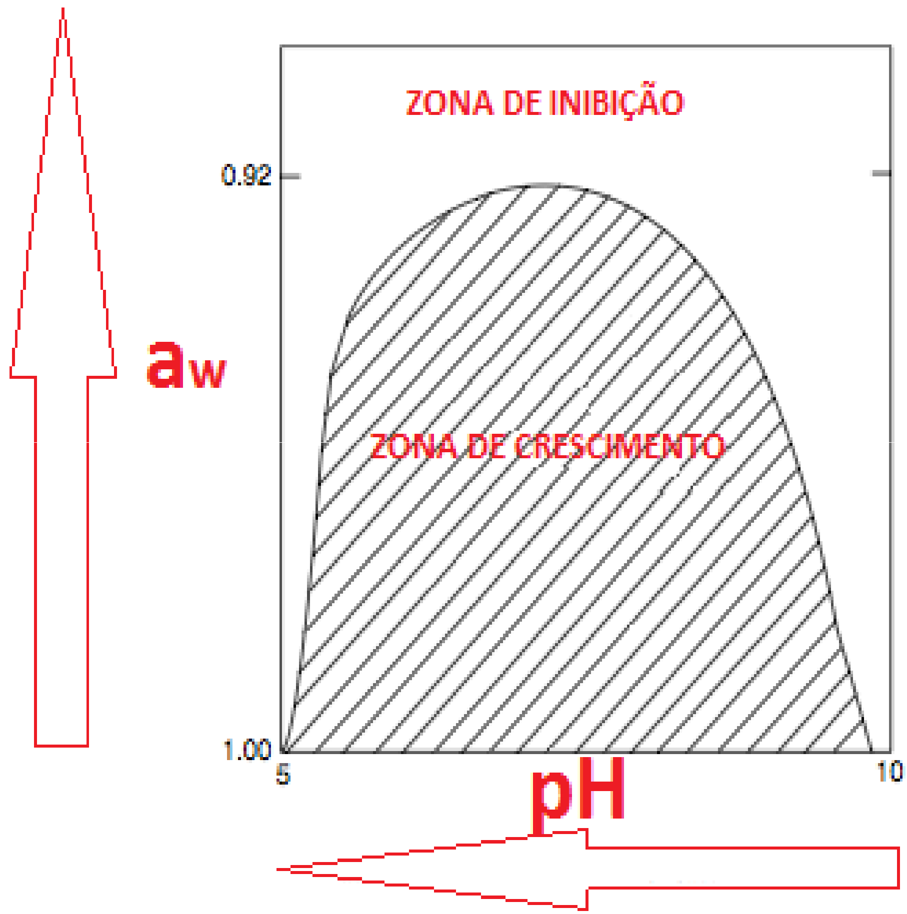
MICROORGANISMS	Minimum pH
Salmonella paratyphy ϕ	4,0
Escherichia coli ϕ	4,4
Pseudomonas aeruginosa	5,6
Vibrio parahaemolyticus ϕ	4,8
Bacillus cereus ϕ	4,9
Clostridium botulinum ϕ	4,7
Staphylococcus aureus ϕ	4,0
Enterococcus spp	4,8
Lactobacillus spp	3,8
Saccharomyces cerevisiae	2,3
Aspergillus oryzae	1,6
Penicillium italicum	1,9

ϕ: microorganisms that represent food hazards.
Adapted from ICMSF(1980).

Table 6. Classes of food products according to their pH.

FOOD PRODUCTS	pH
NEUTRAL	**7,0-6,5**
CARCASS MEAT	
MILK	
HAM	
LOW ACID	**6,5-5,3**
RAW BEEF	
BACON	
CANNED VEGETABLES	
MEDIUM ACID	**5,3-4,5**
FERMENTED VEGETABLES	
PICKLED CUCUMBERS	
MOST CHEESES	
ACID	**4,5-3,7**
FRUITS	
FRUIT JUICES	
TOMATOES	
FERMENTED VEGETABLES	
YOGHURT	
HIGH ACID	**<3,7**
PICKLES	
SAUERKRAUT	
CITRUS FRUITS	

Adapted from ICMSF (1980).



ZONA DE INIBIÇÃO
0.92
aw
ZONA DE CRESCIMENTO
1.00
5
10
pH

ORGANIC ACIDS

- The short chain organic acids such as:
  acetic, benzoic, citric, propionic and sorbic

  are most commonly used as food preservatives or acidulants.

  It is the undissociated molecule of the organic acid that is responsible for its antimicrobial activity.

  The undissociated molecule is readily soluble in cell membranes and interferes with its permeability.

  Lowering the pH of a food increases the proportion of undissociated molecules of an organic acid and thus increases its effectiveness as antimicrobial agent.

  ICMSF (1980)

Table 8. Commonly used organic acids, dose of use and examples of usage.

ORGANIC ACID	Concentration (g/Kg)	Examples of foods
ACETIC ACID	No limits	Pickles
CITRIC ACID	No limits	Soft drinks
SORBIC ACID	0,1-2	Fresh and processed cheese; Jams and jellies; Semi preserved meat and fish products
SODIUM BENZOATE	0,1-2	Pickles; Fruit juices;Jams; Soft drinks
SODIUM PROPIONATE	0,1-3	Bread and bakery; Cheese

Adapted from ICMSF (1980).

Curing salts

- Curing originatelly developed to preserve certain foods by the addition of sodium chloride. Sodium nitrate ($NaN0_3$), a natural impurity of sodium chloride (NaCl), was shown to be responsible for the development of a pink to red pigment in meat. Subsequently it was found that it was nitrite ($N0_2$), formed by bacterial reduction of nitrate, the important compound in the development of colour. Nitrite added to meat is converted to an equilibrium mixture of $N0_3$, $N0_2$ and nitric oxide (No). Nitrate eventually disappears as the result of chemical reactions with components of meat or from the metabolic activity of microbes.

- The precise mechanism of inhibition of bacteria by nitrite is unknown.

  It does not prevent spore generation but prevents outgrowth.

  At the concentrations used (0,13g/kg) it must be regarded as a bacteriostatic agent.

- Ascorbate is important in accelerating the development and stabilization of cured meat pigment. Additionally it increases the anticlostridial activity of nitrite in canned pasteurized meats. As reducing agent ascorbate act as oxygen scavenger, decreases the *Eh*, participates in the reduction of metmyoglobin to myoglobin, reacts with nitrite to increase the yield of nitric oxide and it also chelates pro-oxidants such as copper and iron. Ascorbate probably do not has antimicrobial activity and, its role results from its ability to increase the antimicrobial activity of nitrite.

Types of meat products according to its shelf stability.

Adapted from Norman & Corte (1985) and FAO (1990).

Category criteria	Water activity/pH	Storage temperature	Examples
HIGLY PERISHABLE	a_w > 0,95; pH>5,2	+ 5ºC	Fresh Meat Cooked Ham
PERISHABLE	a_w <0,95; pH<5,2	+ 10ºC	Semi dry sausages Carne de Sol
SHELF STABLE	a_w <0,95 and pH<5,2 or only a_w < 0,91	No refrigeration required	Dryed meats Cured raw ham Charque


LEVONI
Parma
Jambon Trevelez
Espagne
100 g 6.75
Jambon Pata Negra
Porcs nourris exclusivement avec des glands
Espagne 100g 15.-
Elevage en Semi-Liberté
Bresaola
Italie
100 g 6.95
Jambon cru
San Daniel
Italie
100 g 7.99
Jambon de Parme
Italie
100 g 7.95
Viande séchée du Valais de boeuf
100 g 7.50
iande séchée
e cheval
arada
100 g 5.15

Oxidation reduction potential (*Eh*)

- Redox potential is an important selective factor in all environments including foods and probably influences the types of microbes found and their metabolism.
- Although *Eh* is not a important processing parameter in food manufacture it undoubtedly interacts with other factors such as pH and gaseous atmosphere to determine the spoilage microflora of many foods. Reduced *Eh* levels (+10 to -130 mV) prevent the growth of aerobic microorganisms but may encourage the growth of Enterobacteria and Clostridia. Vegetables have *Eh* values from +300 to +400 mV and are consequently spoiled by aerobic bacteria and molds.

Smoke

- Smoking was an important component of the preservation process of many cured meat and fish products. Today is important to aid the preservation of only a few products and it is mainly used to contribute to colour and flavour.

- Smoke contains a wide variety of organic compounds including:

  antibacterial phenolic compounds

  formaldehyde

Starter cultures

- Starter cultures are used in many types of fermented foods to bring about a more rapid and complete fermentation than may occur from the indigenous microflora.
- In developed countries, starter cultures are widely used by the dairy and meat industry in products such as cheeses, yoghurt, fermented milks and sausages.

Combination of factors: Hurdle Technology

- Hurdle technology is used in several countries for the gentle but effective preservation of foods. Previously hurdle technology, *i.e*., a combination of preservation methods, was used empirically without much knowledge of the governing principles. Since about 20 years the intelligent application of hurdle technology became more prevalent, because the principles of major preservative factors for foods (*e.g*., temperature, pH, *a*w, Eh, competitive flora), and their interactions, became better known Leistner (2000).

- The physiological responses of microorganisms during food preservation (*i.e.,* their homeostasis, metabolic exhaustion, and stress reactions) are the basis for the application of advanced hurdle technology. The disturbance of the homeostasis of microorganisms is the key phenomenon of food preservation. The goal for an optimal food preservation is the multitarget preservation of foods, in which intelligently applied gentle hurdles will have a synergistic effect (Leistner, 2000).

- The most important hurdles used in food preservation are temperature, water activity (*a*w), acidity (pH), redox potential (*Eh*), preservatives (*e.g*., nitrite, sorbate, sulfite), and competitive microorganisms (*e.g.,* lactic acid bacteria). However, more than 60 potential hurdles for foods, which improve the stability and/or quality of the products, have been already described (Leistner, 2000).

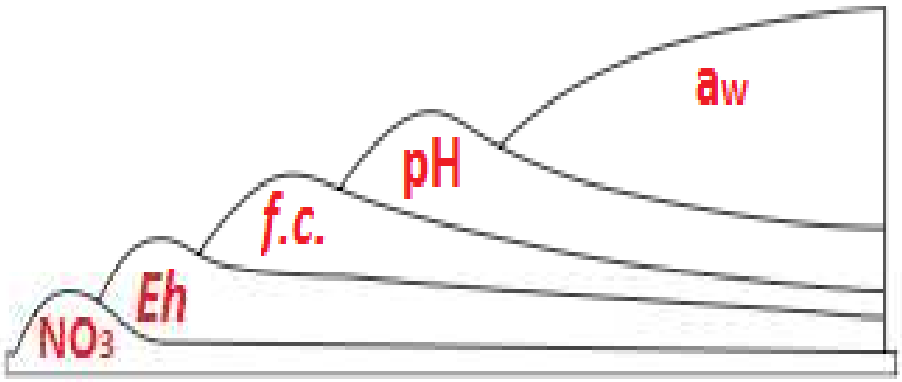


FIGURE 36.2 Sequence of hurdles occurring during the ripening and drying of fermented sausages (salami). Symbols have the following meaning: pres., addition of nitrite-curing-salt; Eh, decrease of redox potential; c.f., growth of competitive flora; pH, acidification; a_w, decrease of water activity during the drying process. (After L. Leistner, *Food Design by Hurdle Technology and HACCP*, Adalbert-Raps-Foundation, Kulmbach, 1994, p. 62; L. Leistner, in *Water Activity: Theory and Applications to Food* Marcel Dekker, Inc., New York, 1987, p. 295.)