IQRO JURNALI / 2023
GUVOHNOMA № 060680
E-ISSN : 2181-4341

VOL–2 ISSUE–1
https://wordlyknowledge.uz/

UDC: 5995-579-579.0

ORCID:0000-0001- 5268-3948

THE EFFECT OF FOOD STRUCTURE ON MICROBIAL ACTIVITY

Musurmonov Abror Alisherovich

Choriyev Jahongir Olimjon o'g'li

Denau Institute of Enterpreneurship and Pedagogy Biology students

Ubaydullayeva Shohista Hidoyatillo qizi

Termiz Institute of Agrotechnologies and innovative Development

Quarantine direction of plants and agricultural products student

Email: usmonovkamol0@gmail.com

Annotation: The ability of microorganisms to grow on foods depends on storage conditions, food composition, presence of additives and food structure. But, in structured foods the mobility is restricted; microorganisms are immobilized and grow as colonies. It must be considered that most of food products present some degree of structure, such as the case of emulsions, gels and solid foods. However the effect of structure on microbial growth is scarce evaluated specially when dealing with spoilage flora. The main objective of this chapter is to review the bibliography concerning the effect of structure on microbial growth and on the activity of stress factors with special emphasis on water activity (a_w) depressors and on antimicrobial agents. This information will help to choose the conditions that assure food microbial stability and contribute to improve the food safety and quality.

Key words: Food structures: ready and natural emulsions, vegetables, fruit structures.

INTRODUCTION

Foods are in general dispersed systems and most of them exhibit a structure. The latter is provided by the presence of vegetal or meat tissues or by the inclusion of hydrocolloids and lipids in order to get viscous, gelled or emulsified food products. Some examples of the main structuring agents used in food products are detailed in Table 1.

The ability of microorganisms to grow on foods depends on storage conditions, food composition, presence of additives and food structure. Food structure modifies water mobility and distribution of solutes such as acidulants, a_w depressors and preservatives. Furthermore, it affects the mobility of microorganisms. It is well known that the site of microbial growth is the aqueous phase and that in liquids, it occurs planktonically. The medium surrounding microorganisms is uniform; transport of nutrients to the cell occurs freely and also the metabolites produced during growth are able to diffuse into the medium. On the contrary, in structured foods the mobility is restricted; microorganisms are immobilized and grow as colonies. As a result of the close spatial distribution, colonies can compete for nutrients and oxygen; besides, their metabolic end products can be accumulated near colonies affecting growth. Moreover, susceptibility to stress factors is modified.

As previously mentioned, most food products present some degree of structure, such as the case of emulsions, gels and solid foods. However, the effects of structure on microbial growth and on the effectiveness of stress factors have been partially evaluated specially when dealing with spoilage flora. Briefly, trends reported about the effect structure on microbial growth are diverse. Many studies postulate that structure acts as an additional stress factor and therefore lower growth is expected. For example, Meldrum et al. found that *Listeria monocytogenes* in a gel made with gelatin grew more slowly than in broth. Brocklehurst reported that a_w depression with sodium chloride in a gelatin gel was more effective than in broth for decreasing the growth rate of *Bacillus cereus*. Conversely, other studies show that structure increases growth probability. For example, Wilson et al. reported that the addition of sucrose to broth promoted a decrease in *Staphylococcus aureus* growth, but when sucrose was added to a gelatin gel, *S. aureus* growth rate remained unaffected. The main objective of this chapter is to review the bibliography concerning the effect of structure on microbial growth and on the activity of stress factors with special emphasis on a_w depressors, pH adjustment and on antimicrobial agents. This information will help to choose the conditions that assure food microbial stability and contribute to improve the safety and quality of foods.

Table 1. Examples of structuring agents present in food

Structure agent	**Food**
Hydrocolloids with gelling ability(polysaccharides and proteins)	Jams, marmalade, dairy and fruit based desserts, jellies, cream cheeses, pates
Oil in water	Salad dressings, sauces, milk products containing cream
Water in oil	Butter, margarine, fat spread
Cellulose, Hemicelluloses and pectins	Vegetable tissues
Miofibrillar proteins	Meat tissues

EFFECT OF GELS ON MICROBIAL GROWTH AND ONTHE ACTIVITY OF STRESS FACTORS

Gels represent one of the simplest structured systems. Gelling agents are incorporated into food formulations in order to modify their microstructure and texture .However, the effects may be wider. It must be noted that in gelled products, microorganisms are immobilized and grow as micro-colonies Mentioned immobilization affects bacterial growth rate, as well as microbial response to environmental conditions, as a consequence of the reduced metabolic activity found in some regions of the colony. Compared to cells in suspensions, bacteria immobilized in solid media have to overcome an additional stress to be able to initiate their growth. The lower microbial growth observed in solid than in liquid media may be related to nutrient diffusion, oxygen availability, the rate and profile of end-products production and cell to cell communication .

Gelled foods are obtained by the addition of hydrocolloids which are polysaccharides or

proteins. In addition to their uses as texturizing agents in the food industry, they are used to model solid food products for research purposes. The gelling agents most frequently selected are agar and gelatin. Agar is a polysaccharide obtained from seaweed and gelatin is a fibrous protein derived from collagen. Gelatin has a low melting point (25-37°C), as a consequence, it is possible to inoculate a microorganism at low temperature, without its inactivation. In addition, it is easy to remelt the gel for subsequent analysis. On the other hand, agar solidifies at 45°C and melts at 85°C. The high temperature at the inoculation step may be stressful for microorganisms, but the obtained gel is less influenced by temperature changes. As an advantage, agar is nontoxic and physiologically inert toward microorganisms . Both gelling agents are generally recognized as safe food ingredients. To evaluate microbial growth in model gelled systems, the inoculated gelling agent (mainly agar or gelatin) is placed in Petri dishes, microplates or in a Gel Cassette. The latter consists of a frame holding a layer of gel. Mentioned frame is sealed with a plastic film, which is gas. This system can be used to study microorganism‘s growth on the gel surface as well as within the gel matrix by measuring microbial growth by plate count. Plate count is a time consuming technique. To solve up this problem,

The role of structure on the inhibition of microorganisms‘ growth was widely demonstrated. A compilation of the studies on this subject is shown in Table 2. As an example observed that *L. innocua* growth rate decreased as gelatin concentration increased and that micro-colonies morphology changed as gelatin concentration was varied. However, gelling could not be used as the principal stress factor in food. The combination with other preservation factors is necessary to guaranty food safety. In reference to a_w, it was reported that its depression decreased the growth rate or the maximum population reached by bacteria and that the magnitude of the effect was dependent on the type of solute used. Although the effect of a_w was greater in liquid media, the presence of solutes produced significant changes on bacterial development in gelled systems. As an example, reported that sucrose produced a decrease in *L. monocytogenes* Scott A growth rate in gel cassettes. In addition, Brocklehurst et al. observed that the maximum population reached by *Salmonella* Typhimurium during growth decreased as the concentration of NaCl or sucrose increased. Furthermore, it was shown that lowering a_w from 0.990 to 0.970 produced the elongation of lag phase and the decrease of growth rate and the maximum population reached by *S.* Typhimurium at different pH values or gelatin concentrations Concerning the effect of pH, Meldrum et al observed that the minimum pH at which *L. monocytogenes* Scott A was able to initiate its growth was higher for an immobilized culture than for a planktonic one. Similar results were obtained by Brocklehurst et al.. Moreover, this trend is enhanced at low a_w values. This effect is related to the fact that the presence of gelling agents increases the buffering capacity of the medium, which offers protection to microorganisms .

As regards the combined effect of mentioned stress factors, Koutsoumanis et al. studied the effect of structure, pH and a_w on bacterial growth and observed that the minimum values that allowed the growth in agar were higher than in broth, being even higher when temperature was decreased. Moreover, it has been observed that refrigerated incubation, at low pH, low a_w and immobilization may prevent *L. monocytogenes* Scott A growth and even cause the loss of cell viability .

Table 2. Compilation of studies about the effect of structure on microbialgrowth in gelled systems

Gel	Microorganism inoculated	Additional stress factor	Reference
Gelatin	*S. Typhimurium*	aw	Brocklehurst et al., 1997
Agar	*Zygosaccharomyces bailii*	pH, aw, temperature	Dang et al., 2010
Agar	*L. monocytogenes*	pH, aw, temperature	Koutsoumanis et al.,2004
Gelatin	*L. monocytogenes*	pH, aw	Meldrum et al., 2003
Gelatin	*S.Typhimurium*	pH, essential oil	Skandamis et al., 2000
Agar, Carbopol, carboxymethyl cellulose, gellan gum, locust bean gum, xanthan gum	*Z. bailii*	pH, aw	Mertens et al., 2009
Carbopol, xanthan gum	*Z. bailii*	pH, aw, temperature	Mertens et al., 2011
Gelatin	*S. Typhimurium*	pH, aw	Theys et al., 2010
Gelatin	*Yersinia enterocolitica*	pH	Robins and Wilson 1994
Gelatin	*L. innocua, Lactococcus lactis*	pH	Antwi et al. 2007
Gelatin and dextran	*Escherichia coli*	pH, aw	Boons et al., 2014
Gelatin, xanthan gum and carrageenan	*E. coli, S. Typhimurium*	pH, aw	Boons et al., 2013

EFFECT OF EMULSIONS ON MICROBIAL GROWTH AND ON THE ACTIVITY OF STRESS FACTORS

Oil in Water Emulsions

Many foods are oil in water emulsions from the physical point of view. The oil concentration varies between 3-5% in the case of milk, from 10 to 40% for salad dressings and can be as high as 85% for mayonnaise. The oil phase consists of polydispersed droplets with a diameter of 0.15-10 μm .When droplets concentration is high, the space between droplets can be of the same order as the diameter of the droplets. This trend limits the space available for microorganism to grow .

The study of microbial growth in emulsions is more difficult than in gels due to the fact that emulsions are opaque and microscopy

and absorbance based methods cannot be applied.

To solve up this problem, Parker et al. developed a method that removes the oil phase of the emulsion with a mixture of methanol and chloroform prior to scanning electron microscopy, light microscopy or transmission electronic microscopy evaluation. Using these techniques, they found that *L. monocytogenes* and *Y. entercolitica* grew in the form of colonies in emulsions containing hexadecane (30-83%) or double cream (33% fat) with or without agarose. In the case of dairy cream, it was found that bacteria were associated with particulate material, probably with casein and that little fat content was trapped among bacteria. Many regions of the sample were sterile and bacteria were concentrated in small areas. This fact has to be taken into account when evaluating microbial stability of these products.

Evaluated the effect of hexadecane and sunflower oil concentrations and droplet diameter on the form of *L. monocytogenes* and *Y. entercolitica* growth using the previously described method. They found that when mean droplet was around 2 μm and concentration of oil was high -in order to have close packed droplets-, bacteria grew as colonies and growth rates were lower than in liquid media. The increase in oil droplets to 15-25 μm removed the inhibitory effect on growth rate but the population at the stationary phase remained lower than the one found in liquid media.

Water in Oil Emulsions

Several foods such as butter, margarine and fat spreads are water in oil emulsions in which droplets of aqueous phase (0.30-30 μm) are dispersed in the oil phase. Microbial growth takes place in water droplets therefore space and nutrient availability restrict growth. A smaller droplet size and its separate distribution enhanced microbial stability. Based on commented trends, mechanistic models were develop and applied successfully to predict the potential for bacterial growth based on droplet size and bacterial energy demands. Mentioned models show that

EFFECT OF STRUCTURED FOODS ON MICROBIAL GROWTH AND ON THE ACTIVITY OF STRESS FACTORS

In meat and vegetable tissues growth occurs at the surface. Different model systems manufactured with gelling agents were used to study this issue. As an example, a solid surface made with agar was used to evaluate the effect of gas atmosphere composition on the growth of food-borne pathogens. In surfaces, growth occurs in colonies and constraints on growth were the same as in gels. But, diffusion limitations and accumulation of protons under the colony are greater than in gels; as a consequence, microorganism growth rate becomes lower in surface colonies than in immersed colonies. Furthermore, many foods contain micro-architectures and growth of microorganisms can take place plancktonically, in colonies -immersed or at the surface- depending on the localization of the microorganisms. Water is located within the microstructure and its distribution is not uniform, in this way different a_w values can be found providing a heterogeneous environment for microorganism. As a_w gives information of the global available water, its determination must be complemented with the information of the different populations of water within the structure. The latter can be evaluated using proton nuclear magnetic resonance (NMR). These measurements were done and used to evaluate their relationship with food structure in cheese. In the following sections, the effect of structure on microbial growth in vegetables, dairy products, meat and meat products are discussed.

PREDICTIVE MICROBIOLOGY IN STRUCTURED MEDIA

Predictive microbiology pretends to reduce time-consuming and costs involved in

challenge tests through the model of microbial growth as a function of time (primary models) and as a function of a few environmental factors (secondary models). The last factors include the traditional ones such as temperature, pH, a_w, and others like antimicrobials, organic acids and oxygen. However, sometimes microbial growth cannot be predicted by these models since some factors such as background flora, microbial competition, stress factors, medium structure and environmental changes produced by microbial growth are not taken into account. This omission is the main source of error in predictive microbiology and it is called as the *completeness error*.

Concerning about this, Boons et al. studied the effect of increase complexity of the structured medium on *E. coli* and *S. cerevisiae* growth. They included heterogeneous systems and NaCl as a stress factor mimicking the inhomogeneous composition and structure of foods. Microbial dynamics was affected by medium structure complexity since the microorganisms showed higher growth in complex than in liquid medium. However, the behavior of both microorganisms was different in the same structured medium. A secondary model including the effect of medium structure on S. Typhimurium growth rate, previously developed by Theys et al., was successfully validated in pasteurized milk and cheese. Also, this model described *L. innocua* and *Lb. lactis* growth as a function of gelatin level.

Different models such as Fickian diffusion model, to predict the diffusion of nutrients and metabolites, and Buffering Theory, to predict local pH changes, have being developed to be incorporated into an integrated modeling methodology to predict growth in structured systems. Regarding to this, Van Impe et al. (2013) explain that traditional predictive models consider the behavior of average population and fails in the description of colony dynamics since the local competition for nutrients causes a different behavior of the individual colony cells and does not have a normal distribution. They propose considering the effects of environmental conditions on cell metabolism and growth dynamics by using Metabolic Flux Analysis. They suggest that this information will allow improving precision of predictive models for more complex systems like structured media. More recently, Tack et al. applied an individual based model on *E. coli* growth in gel from cellular parameters reported in bibliography. The model included the local nutrient competition, individual cell differences and intercolony interaction. It successfully reproduced single colony dynamics, simulated interaction between colonies and demonstrated that nutrient diffusion and local cellular glucose competition produced emergence of a starvation zone in the center of the colony. From the knowledge of cellular parameters of microorganisms in structured media, this model contributes to microbiological quality and safety of structured foods

CONCLUSION

Microorganism‘s development in food is not only determined by its environmental and storage conditions but also by its structure. Concerning this influence, the following facts need to be remarked:

Microbial growth in structured systems is inhibited compared to liquid systems. Therefore, the response of microorganisms to different stress factors is modified with respect to planktonic growth and different trends can be observed depending on the stress factor applied, the composition of the system and the microorganism.

Independently of its role as a structuring agent, the presence of oil affects the physiology of microorganism and the distribution of lipophilic additives. Hence, the effectiveness of lipophilic antimicrobial agents can be decreased.

There is some information available about the effect of the structure itself and the stress factors such as pH and a_w in systems modeled by gels. However, this information is scarce in emulsified systems. This trend can be linked with the difficult for studying growth in emulsions due to their opacity which ruled out the use of microscopy and absorbance based methods.

The effect of medium structure has been introduced as a factor in predictive microbiology models in the last years. However, further research is needed in this area.

Many foods contain micro-architectures and growth of microorganisms can take place plancktonically, in colonies -immersed or at the surface- depending on the localization of the microorganisms.

In vegetables, it is essential to understand the factors affecting pathogen attachment in order to apply strategies to avoid the growth. Commented studies demonstrated that attachment ability depends on the pathogen, the surface morphology of the vegetables, the temperature and the integrity of the tissue.

In dairy fermented products, structure determines the location of microorganisms during the processing. The role of microorganisms and their enzymes on ripening processes is a key factor on the quality of these products, underlying the importance of this feature.

In meat and meat products, many are the structures -from fiber structure to meat emulsions- which can exert several effects on microbial growth. Mainly, they can modify the action of preservatives and condition the distribution of compounds in different phases.

Outcomes shown herein highlight the importance of considering the effect of the structure on microbial growth when evaluating microbial stability of these food systems.

REFERENCES:

1.Aasen, I. M., Markussen, S., Møretrø, T., Katla, T., Axelsson, L. and Naterstad, K. (2003). Interactions of the bacteriocins sakacin P and nisin with food constituents. *International Journal of Food Microbiology*, *87*, 35–43.

2.Aguilera, J. M. (2005). Why food microstructure? *Journal of Food Engineering*, *67*, 3-11.

3.Vahobov H.A., Rasulova T.X. Microbiology . Tashkent-2009.

4.Anang, D. M., Rusul, G., Bakar, J. and Ling, F. H. (2007). Effects of lactic acid and lauricidin on the survival of *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli* O157:H7 in chicken breast stored at 4°C. *Food Control*, *18*, 961–969.

5.Antwi, M., Bernaerts, K., Van Impe, J. F. and Geeraerd, A. H. (2007). Modelling the combined effects of structured food model system and lactic acid on *Listeria innocua* and *Lactococcus lactis* growth in mono and coculture. *International Journal of Food Microbiology*, *120*, 71-84.

6.Nazarov O. Microbiogy. Tahkent-2009. 23-26.

7.Antwi, M., Geeraerd, A. H., Vereecken, K. M., Jenné, R., Bernaerts, K. and Van Impe, J. F. (2006). Influence of a gel microstructure as modified by gelatin concentration on *Listeria innocua* growth. *Innovative Food Science and Emerging Technologies*, *7*, 124-131.

8.Baka, M., Noriega, E., Tsakali, E. and Van Impe, J. F. M. (2015). Influence of composition and processing of Frankfurter sausages on the growth dynamics of *Listeria monocytogenes* under vacuum. *Food Research International*, *70*, 94–100.

9.Mustaqimov G.D. Plants and microbiology. Tashkent , “O’qiyuvchi”-1998.

10.Belitz, H. D. and Grosch, W. (2009). Food Chemistry: Milk and Dairy Products (4ª edition).

Berlin Heidelberg, Springer-Verlag.

Bennik, M. H. J., Smid, E. J., Rombouts, F. M. and Gorris, L. G. M. (1995). Growth of psychrotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. *Food Microbiology*, *12*, 509-519.

11.Bhatti, M., Veeramachaneni, A. and Shelef, L. A. (2004). Factors affecting the antilisterial effects of nisin in milk. *International Journal of Food Microbiology*, *97*, 215-219.

12.Blagojevic, B., Antic, D., Adzic, B., Tasic, T., Ikonic, P. and Buncic, S. (2015). Decontamination of incoming beef trimmings with hot lactic acid solution to improve microbial safety of resulting dry fermented sausages - A pilot study. *Food Control*, *54*, 144–149.

13.Boons, K., Noriega, E., Van den Broeck, R., David, C. C., Hofkens, J. and Van Impe, J. F. (2014). Effect of microstructure on population growth parameters of *Escherichia coli* in gelatin-dextran systems. *Applied and Environmental Microbiology*, *80*, 5330-5339.

14.Boons, K., Noriega, E., Verherstraeten, N., David, C. C., Hofkens, J. and Van Impe, J. F. (2015). The effect of medium structure complexity on the growth of *Saccharomyces cerevisiae* in gelatin-dextran systems. *International Journal of Food Microbiology*, *199*, 8-14.

15.Boons, K., Van Derlinden, E., Mertens, L., Peeters, V. and Van Impe, J. F. (2013). Effect of immobilization and salt concentration on the growth dynamics of *Escherichia coli* K12 and *Salmonella* Typhimurium. *Journal of Food Science*, *78*(4), M567-M574.

16.Bosilevac, J. M., Nou, X., Barkocy-Gallagher, G. A., Arthur, T. M. and Koohmaraie, M. (2006). Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and Enterobacteriaceae and reduce the prevalence of *Escherichia coli* O157:H7 on preevisceration beef carcasses. *Journal of Food Protection*, *69*, 1808–1813.

17.Brocklehurst, T. (2004). Challenge of food and the environment. In R. C. McKellar, and X. Lu (Eds.). *Modeling Microbial Responses in Food*. London, CRC Press, Francis and Taylor Group.

18.Brocklehurst, T. F., Mitchell, G. A. and Smith, A. C. (1997). A model experimental gel surface for the growth of bacteria on foods. *Food Microbiology*, *14*, 303-311.

19.Brocklehurst, T. F., Parker, M. L., Gunning, P. A., Coleman, H. P. and Robins, M. M. (1995). Growth of food-borne pathogenic bacteria in oil-in-water emulsions: II. Effect of emulsion structure on growth parameters and form of growth. *Journal of Applied Bacteriology*, *78*, 609–615.

20.Brocklehurst, T. F., Parker, M. L., Gunning, P. A. and Robins, M. M. (1993). Microbiology of emulsions: *Physicochemical aspects. Lipid Technology*, 83–88.

21.Brocklehurst, T. F. and Wilson, P. D. G. (2000). The role of lipids in controlling microbial growth. *Grasas y Aceites*, *51*, 66–73.

22.Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology*, *94*, 223–253.