PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 15 (3): 249-254, 2016
ISSN 1680-5194

A Microstructure of the Modeling Systems on the Basis of the Fermented Raw Material

Zinina Oksana Vladimirovna[1], Rebezov Maksim Borisovich[1] and Vaiscrobova Evgeniya Sergeevna[2]
[1]South Ural State University (National Research University),
Chelyabinsk, 454080, Lenin Prospekt, 76, Russia
[2]Nosov Magnitogorsk State Technical University, Chelyabinsk Region,
455000, Magnitogorsk, Lenin Avenue, 38, Russia

Abstract: A theoretical and practical interest to the expansion of the use of collagen containing raw material field in the meat foods production has been existing for many decades. But this raw material is notable in a technological respect for the unacceptable qualities that it is possible to improve by means of the biotechnological processing of the raw material. In recent years the propionic and bifidus bacteria have been drawing many scientists' attention for the ferment processing conducting of the meat raw material. The propionic and bifidus bacteria have a high proteolytic activity and protective qualities in respect of the pathogenic and opportunistic pathogenic microflora. In this field scientists' efforts are concentrated on the use of the mentioned bacteria in the production of fermented sausages or for the low-grade raw material softening. An aspect of using of the propionic and bifidus bacteria is studied not enough for the collagen containing raw material processing. Thereby the aim of the work is the study of the influence of the ferment processing of the collagen containing by-products of the 2nd category of bovine animals on the model systems micro structure, including protein compositions on the basis of the biomodificated by-products. In the study a detailed description of the change of the particular structural components of a model system is given when including to the model system the biomodificated raw material. It is established that under the influence of the ferment processing the decondensation of collagen fascicles occurs to particular fibrils and their fragmentation takes place. As a result when making protein compositions the homogeneous in structure emulsion comes out. Later on it is recommended to use the emulsion in boiled sausages and minced half-finished goods receipts.

Key words: Bifidus bacteria, propionic bacteria, by-products, collagen, micro structure

INTRODUCTION

Collagen is a part of the structure of almost every tissue of animals. It supports the functions of organs and is one of the important components of the animal raw materials conversion products under industrial enterprises conditions.

Collagen is not an individual protein, but a family of similar proteins with some structural differences depending on an anatomical function and organism species (Rivier and Sadoc, 2007).

Collagen exists in several forms. The main structure of all types of collagen is similar (Eis, 1964; Wess, 2008). A molecule of collagen is composed of a triple dexiotropic helix, which generally consists of three identical α-chains. Such formation is known as the tropocollagen (Eis, 1964; Asghar and Henrickson, 1982; Djabourov *et al*., 1993; Boot-Handford and Tuckwell, 2003; Gomez-Guillen *et al*., 2011). One spiral turn of a-chain contains three amino-acid residues. Ladder-shaped parallel rows of the tropocollagen molecules compound the source of a structural organization of a collagenic fiber. The tropocollagen molecules are lengthwise and crosswise directed and are displaced to a quarter that causes a cross striation of fibrils (Eis, 1964; Wess, 2008).

A collagen structure complicacy defines important functional characteristics of this protein (Eis, 1964; Asghar and Henrickson, 1982; Djabourov *et al*., 1993; Boot-Handford and Tuckwell, 2003; Rivier and Sadoc, 2007; Wess, 2008; Gomez-Guillen *et al*., 2011) using in the technology of animal tissues conversion:

- An ability to keep the structure on the molecular level when separating from the subtend components
- An ability after an exudation and conversion to the solution to a modification with the formation of supramolecular structures, that is widely used by producing artificial collagenic materials.
- A possibility of the supramolecular structure stabilization and its additional structuring being the basis of the conservation, an elementary raw material conversion containing collagen and also producing artificial or modified collagenic materials

Corresponding Author: Zinina Oksana Vladimirovna, South Ural State University (National Research University), Chelyabinsk, 454080, Lenin Prospekt, 76, Russia

Collagen is taken a poor effect by a digestive enzymes induction (Asghar and Henrickson, 1982; Gomez-Guillen *et al*., 2011). An it's qualified as a protein of a low biological value because of the most important amino acid lack in the structure. However a collagen role in the feeding has been recently revised. According to a physiological effect it's reckoned among the dietary fibers-necessary components of adults and children dietary intakes.

There is a theoretical and practical interest to the expansion of the field in using the containing collagen raw material in meat foods production many decade years. In a modern technology of the animal raw material conversion basic principles of the containing collagen raw material appliances are formed, allowing to level negative characteristics of its formation and qualities:

- Changing the structure of the containing collagen raw material by means of its reduction, an embodiment of hydrolysis in solution of nutritional acids and alkalis, thermal treatment conduction (one-time or multiple), that is allowed to improve organoleptic characteristics of raw material considerably, to improve a function- technological potential (a swelling capacity, a gel-forming and water-bound ability) (Djabourov *et al*., 1993), to prevent an undesirable microflora development and oxidizing process of lipids
- The use of the containing collagen raw material together with commercial protein preparations, which on the one hand compensate the lack of essential amino acids, on the other hand provide a display of fat retained and emulsion qualities by systems on basis of the containing collagen raw material under the condition of high structure and water-bound characters of hydrothermic decomposition of collagen products (glutin, gelatosis, gelatin)
- A quantitative introduction of the containing collagen raw material, as a rule, is limited 12-15% from the whole content of protein in meat foods and since collagen can perform a function of fiber intake, its presence is physiologically grounded in the present concentration (Rivier and Sadoc, 2007)
- An improving of the containing collagen raw material qualities by means of processing enzymatic preparations and starter cultures, that makes it possible to improve totally organoleptic and function-technical qualities, sanitary-microbiological results of raw material. In addition it makes it possible to improve a biological value of raw material at the expense of an essential amino acids accumulation

It should be admitted that the processing of the containing collagen raw material with biologically active substances on basis of life activity products is one of the perspective direction.

It is established that microorganisms change a substrate structure by means of enzymes making new substances that favour the improving of a product qualitative measure.

It is noted by Misra and Kuila (1995) that certain leaven microorganisms are capable to improve a biological value of fermented products due to a biosynthesis of ferments, vitamins, free amino acids.

It is noted by many scientists the next positive moments occurring with the raw material by fermentation:

1: A keeping quality of the raw material is improved at the expense of forming abscopal metabolites such as organic acids (lactic acid, ethanoic, formic, propionic acid), ethanol, bacteriocyns and etc. (Adams, 2008)
2: A sanitary and hygienic condition of the raw material is improved at the expense of inhibition and even an exclusion a pathogen from it (Fujiwara *et al*., 1997; Caplice and Fitzgerald, 1999; Biavati *et al*., 2000; Yuksekdag *et al*., 2014)
3: A polymer assimilability is improved (Boot-Handford and Tuckwell, 2003)
4: A nutritional substrates enrichment is occurred with nutritional supports (with vitamins, proteins and essential amino acids, fatty acid and etc. and an improving of a biological value of nutritional components is occurred at the expense of nutritional matrix catabolism (Hao and Scherada, 1994; Jones and Miller, 1991)
5: Organoleptic characteristics are improved, a texture, colour and taste are formed at the expense of the producing flavor-aromatic combinations by microorganisms (Kujawski *et al*., 1996)

In recent years for biotechnological implementation processing of the containing collagen raw material many scientists have been attracted attention by bifidus bacteria and propionic bacteriums that have a high proteolytic activity (Steinkraus *et al*., 2004).

It should be rated to positive qualities of bifidus bacteria, being of an importance in a technology of an animal raw materials conversion:

- An ability to produce the lactic acid and volatile fatty acids (Misra and Kuila, 1995; van Boekel *et al*., 2010)
- A potential ability to reduce a content of the residual sodium nitrite and to stabilize a meet food colour at the expense metabolite, forming in a fermentation carbohydrates process and having reducing characters and also at the expense of reducing an oxidation-reduction potential of a meat system (Misra and Kuila, 1995)
- A high antagonist activity against a pathogenic and opportunistic pathogenic microflora (Geisen and Holzapfel, 1996; Fujiwara *et al*., 1997)

Propionic bacteriums have the next characteristics:

- Stimulate a growth of a bifidobacterium flora, synthesize a wide spectrum of antibacterial components, active to enterobacteriums, putrefactive bacteriums, fungous (Foschino, 1988)
- Take part in a carbohydrates fermentation, alongside with it propionic and acetic acids are cumulated, which block a multiplication of pathogenic germs (Yuksekdag *et al*., 2014)
- Have an influence on the generation and digestion of vitamins, especially B_{12}

The presence of growth substances is of great importance for a normal growth and development of bifidus bacteria. Vitamins (pantothenic acid, biotin, riboflavin), mineral substances (iron, cobalt, magnesium, phosphorus, potassium), vegetable components (skim soya beans, cane sugar, potato extract, carrot) are used as growth-stimulating substances.

It was note by Yuksekdag *et al*. (2014), Coussement and Franck (2001) that lactulose and oligosaccharides are effective growth factors of bifidus bacteria. Vegetable extracts contain biologically active substances, which are nutritional substrates for probiotic microorganisms, that is very important for their growth and metabolism (Antila, 1954; Coussement and Franck, 2001; Cruz *et al*., 2010).

Methods of a microstructured analysis are widely used for an objective and informative detection of both qualitative characteristics of the raw material and their changes under the influence of bacterial culture, for an assessment of an enzymatic processing impact depth upon the structural components changes of animal tissues. The methods are defined as a high degree of an objectivity instead of physicochemical methods that is proved their appliance. Fine-structural investigations make it possible to judge both by the whole product structure and the changes happening in particular structural components, to differentiate characteristics of different tissue and cellular structures (Aktas and Kaya, 2001; Rawdkuen and Benjakul, 2012).

MATERIALS AND METHODS

According to an above-stated material, the aim of the work is a research of the influence of biotechnological processing realized by means of bringing bacterial preparations of bifidus bacteria and propionic bacteriums in the containing collagen minced raw material upon a microstructure of model systems, having got on the basis of the given raw material.

Model systems, having got on the basis of biomodificated containing collagen by-products of the 2nd category of bovine animals-lips and ears are objects of the research. The next start cultures were used for a biomodification:

- Propionic Culture PS-4и Probio-Tec BB-12-bacterial starters frozen-dried of a direct application made by <<Chr.Hansen A/S>> company (Denmark). The given bacterial cultures contain: Propionic Culture PS-4-propionic bacteriums, Probio-Tec BB-12-bifidus bacteria *Bifidobacterium animalis*
- Concentrate Bifilact-Pro, produced by Federal State Unitary Enterprise "An Experimental Biofactory" of Russian Agricultural Academy (Uglich town). The concentrate consists of lactic acid, propionic and bifidus bacteria of *Lactococcus lactis* sub spp. *diacetilactis*, *Streptococcus thermophilus*, *Propionibacterium freudenreichii*, *Bifidobacterium Bifidum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis* ssp

Lactulose syrup and linseed flour were used as growth stimulating substances.

Model systems were got in the following way. Defatted and washed containing collagen by-products were minced by a chopper with a diameter of rack gaps 2-3 mm. A bacterial concentrate Bifilact-Pro was activated in advance sterilized milk at the temperature 37±1°C during 4±2 h. Start cultures PS-4 and BB-12 taking in ratio 1:2 were hydrated in water at the temperature 37°C till the complete dissolution. For the further conduction of a biotechnological processing protein compositions were made from a prepared containing collagen raw material, bacterial preparations and growth stimulating substances:

		Symbols of a protein composition		
	Components of a protein composition	1	2	Control
A part of a component in a protein composition	Minced by-products	10	10	10
	An activated concentrate Bifilact-Pro	2	-	-
	A hydrated mixture of started cultures PS-4 and BB-12 (1:2)	-	2	-
	Linseed flour+Lactulose syrup (1:2)	1	1	1

The obtained protein compositions were mixed till an even distribution of the components and kept at the temperature 4±2°C during 16-24 h. Model systems similar to the sausage meat or minced semi-finished products composition were composed on the base of minced beef and the obtained protein compositions in ratio 60:40. As a control sample a model system was taken on the basis of minced beef and raw by-products, having put to a delicate blending to with the same ratio of the components.

Changes taking place in tissues under the influence of a biotechnological processing were determined with the method of a histological study of the samples. During the test the samples were brought to a destructive condition with the method of burning, chemical agent processing. After the sampling a fixation of the samples in 10% water solution of neutral formalin was conducted,

then it was washed with cold water, compacted in gelatin. The blocks were cut and compacted in 20% formalin solution during 12 h after the cooling of gelatin solution with the samples. Then the blocks were washed, the pieces were cut of 15 x 15 x 4 mm and the sections were made on a freezing microtome. A section was put on a glass slide treated with an ovalbumin and glycerol. The sections were painted with hematoxylin-eosin. Prepared histological preparations were examined under a light microscope Leika DM 1000.

In the process of the test the samples were brought into a destructive state according to the method of burning, a chemical agents processing.

The main means of a measurement, using for the tests are: a freezing microtome N°55, a microscope Leika DM 1000.

Histological sections were painted by hematoxylin-eosin and the changes were exposed after a biomodification of some structural elements in zooming x 200.

RESULTS AND DISCUSSION

Collagen, like another proteins, is ampholyte (Eis, 1964; Asghar and Henrickson, 1982). Lactic and propionic acids are formed in the process of bifidus bacteria and propionic bacteriums vital activity (Biavati *et al*., 2000). A depression of carboxyl groups dissociation occurs under the influence of an acid processing, the main groups of the protein are ionized, i.e., the protein gets a positive charge in general, electrostatic repulsive forces are appeared as a result.

A predominance of the same sign charges causes the finite groups repulsion of side chains. It promotes facilitation of an electrostatic interaction of water dipoles with charged protein centers, i.e., a swelling capacity increasing.

The presence of acids brings to an irregular distribution of ions inside the proteins and environment, that causes an appearance of osmotic forces.

Considerable stresses occur under a summarized action of electrostatic and osmotic phenomena in the structure of collagen. It brings to an abatement and partial break of some bonds, as a result a swelling and aeration (loosening) of the structure of collagen are happening.

This phenomenon is confirmed by the results of a microstructure analysis.

In the study of a control sample structure It was ascertained that a microhistostructure of the minced meat is homogeneous, tightly-friable. The most part of the product composition is a fine-grained protein mass with distributed evenly throughout the sample of particular fragments from fibers and fascicles of a cross-striated skeletal and nonstriated musculature and also the components of fatty tissue. Muscular fibers myoblasts of a skeletal musculature are covered with sarcolemma, that is consisted of collagen and elastic fibers. Muscular fascicles adjoin to each other closely, contain peripheral muscular nuclei in quantity, a well-marked longitudinal and crosscut striation, exposed little to a destruction. Nonstriated muscles strata have an ill-defined fragmentation in some places. Besides, in some cases preserved interstices of blood and lymphatic vessels, nerve endings are found. Conglomerates from fascicles of a friable connective tissue occur in some places. Collagenic fibers are well-marked, ill-destructured.

As a result of the conducted microstructure researches of test samples it was determined that in a biomodification process marked changes of a histological structure of model systems have taken place under the influence of propionic bacteriums and bifidus bacteria in the comparison with a control sample. Histocuts of test samples have a well-marked structural arranging, typical for sausage goods. A minced meat structure is homogeneous, tightly-friable, slightly vacuolated. The most part of the product composition is a fine-grained protein mass with distributed evenly throughout the sample of particular fragments from fibers and fascicles of a cross-striated and nonstriated musculature (Fig. 2a), having a well-marked destruction and defragmentation degree (Fig. 1b). Muscular fibers myoblasts are covered with a connective tissue, subtle, thin-walled sarcolemma, that is consisted of collagen and elastic fibers. Fascicles from collagen fibers of a connective tissue and a nonstriated muscles tissue are considerably separate, destructive and decayed to fibers into components (Fig. 1c)

A degree of a histostructure change depended little from the sort of the starter. The picture of structure changes is almost the same, but to a considerable degree it is expressed in a model scheme N°1 with a protein composition, including the bacterial concentrate Bifilact-Pro. This fact could be explained by a higher proteolytic activity of the concentrate. The higher proteolytic activity is due to the fact that the lactic-acid bacteriums Lactococcus lactis subsp. Diacetilactis are included to the composition of the concentrate besides bifidus bacteria and propionic bacteriums. The Lactic-acid bacteriums Lactococcus lactis subsp. Diacetilactis favoured a more active growth of the bifidus bacteria and also an extra influence of the produced organic acids (first of all the lactic and propionic acids) on the structure of the collagen containing by-products.

Ascertained micro structural changes accompanied a consistency softening of bio modified containing collagen by-products being included in a protein composition. The ascertained micro structural changes accompanied also a decondensation of parenchymatous and interstitial elements, an improvement of organoleptic indexes.

The results of micro structural researches show that by a bio modification of the containing collagen raw material considerable changes occur in the structure of

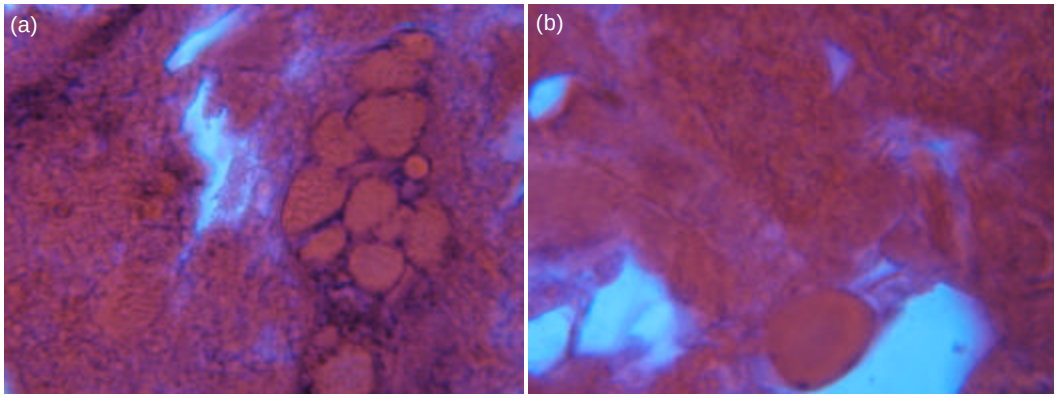

Fig. 1: A histocut of a control sample zoomed x 200

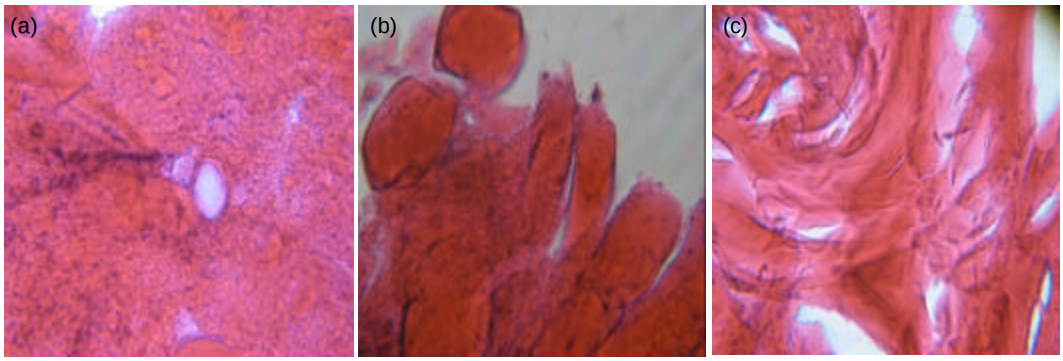

Fig. 2: Histocuts of test samples zoomed x 200

the connective tissue-a decondensation of the collagen fascicles to particular fibrils and their fragmentation. The reason for that is the discharge in the process of a vital activity bifidus bacteria and propionic bacteria of the lactic and propionic acids, which favour the raw material softening. A destructive effect of the lactic acid on the animal tissue was established by Aktas and Kaya (2001). A beneficial action of an enzymatic processing upon an animal tissues microstructure was established by Rawdkuen and Benjakul (2012). In the result of the literary review conduction it was not revealed by any researches concerning an establishment of the model systems microstructure on basis of the ferment by-products of bovine animals with the use of starts of bifidus and propionic bacteria for the fermentation.

It was also ascertained that a homogeneous in structure emulsion comes out when making the protein compositions. Subsequently it is recommended to use the emulsion in boiled sausages and minced half-finished goods receipts.

REFERENCES

Adams, M.R., 2008. Review of the sensitivity of different foodborne pathogens to fermentation. Food Control., 8: 227-239.

Aktas, N. and M. Kaya, 2001. The influence of marinating with weak organic acids and salts on the intramuscular connective tissue and sensory properties of beef. Eur. Food Res. Technol., 213: 88-94.

Antila, M., 1954. Uber die Propionsauretacterien in Emmentaler. Kase Meijerit. Aikakausk, 16: 124-130.

Asghar, A. and R.L. Henrickson, 1982. Chemical, biochemical, functional and nutritional characteristics of collagen in food systems. Adv. in Food Res., 28: 232-372.

Biavati, B., M. Vescovo, S. Torriani and V. Bottazzi, 2000. Bifidobacteria: history, ecology, physiology and applications. Ann. Microbiol., 50: 117-131.

van Boekel, M., V. Fogliano, N. Pellegrini, C. Stanton, G. Scholz, S. Lalljie, V. Somoza, D. Knorr, P.R. Jasti and G. Eisenbrand, 2010. A review on the benefcial aspects of food processing. Mol. Nutr. Food Res., 54: 1215-1247.

Boot-Handford, R.P. and D.S. Tuckwell, 2003. Fibrillar collagen: the key to vertebrate evolution? A tale of molecular incest. Bioessays, 25: 42-51.

Caplice, E. and G.F. Fitzgerald, 1999. Food fermentation: role of microorganisms in food production and preservation. Int. J. Food Microbiol., 50: 131-149.

Coussement, P. and A. Franck, 2001. Inulin and oligofructose. In C.S. Sungsoo and M.L. Dreher (Eds.), Handbook of Dietary Fibre, 721-735.

Cruz, A.G., R.S. Cadena, E.H.M. Walter, A.M. Mortazavian, D. Granato and J.A.F. Faria *et al*., 2010. Sensory analysis: relevance for prebiotic, probiotic and symbiotic product development. Comprehensive Rev. in Food Sci. and Food Safety, 9: 358-373.

Djabourov, M., N. Bonnet, H. Kaplan, N. Favard, P. Favard and J.P. Lechaire *et al*., 1993. 3D analysis of gelatin gel networks from transmission electron microscopy imaging. J. De Physique II, 3: 611-624.

Djabourov, M., J. Lechaire and F. Gaill, 1993. Structure and rheology of gelatin and collagen gels. Biorheol., 30: 191-205.

Eis, A., 1964. The Macromolecular Chemistry of Gelatin. Academic Press, New York.

Foschino, R., 1988. Propionic bacteria activity in different culture conditions. Ann. Microbiol., 38: 207-222.

Fujiwara, S., H. Hashiba, T. Hirota and J.F. Forstner, 1997. Proteinaceous factor (s) in culture supernatant fluids of bifidobacteria which prevents the binding of enterotoxigenic *Escherichia coli* to gangliotetraosylceramide. Appl. Environ. Microbiol., 63: 506- 512.

Geisen, R. and W.H. Holzapfel, 1996. Genetically modifed starter and protective cultures. Food Microbiol., 30: 315-324.

Gómez-Guillen, M.C., B. Gimenez, M.E. Lopez-Caballero and M.P. Montero, 2011. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocolloids, 25: 1813-1827.

Hao, M.U. and H.A. Scherada, 1994. Statistical thermodynamics of protein folding: sequence dependence. J. Phis. Chem., 98: 9882.

Jones, E.Y. and A. Miller, 1991. Analysis of structural design features in collagen. J. Mol. Biol., 218: P.21.

Kujawski, M., L. Lemke, Z. Bator, J. Rymaszewski and G.M. Ciehosz, 1996. Mozliwosci wynorzystania productow frmentacii propionowoej do utrwalania wendlir. Acta Acad. Agric. Ac. Technol. Dsten. Technol. Aliment, 29: 115-129.

Misra, A.K. and R.K. Kuila, 1995. Antimicrobial substances from Bifidobacterium bifidum. Ind. J. Dairy Sci., 48: 612-614.

Rawdkuen, S. and S. Benjakul, 2012. Biochemical and microstructural characteristics of meat samples treated with different plant proteases. Afr. J. Biotechnol., 11: 14088-14095.

Rivier, N. and J.F. Sadoc, 2007. Topology in Molecular Biology. Springer Berlin Heidelberg, 147-162.

Steinkraus, K.H., Y.H. Hui, L. Meunier-Goddik, A.S. Hansen, J. Josephsen, W.K. Nip, P.S. Stanfeld and F. Toldra, 2004. Origin and History of Food Fermentations. Hand-book of Food and Beverage Fermentation Technol., 1-9.

Wess, T.J., 2008. Collagen Structure and Mechanics, Springer US, 49-80.

Yuksekdag, Z.N., D.O. Derilmaz and Y. Beyatli, 2014. Dairy propionibackterium strains with potential as biopreservatives against foodborne pathogens and their tolerance-resistance properties. Eur. Food Res. Technol., 238: 17-26.