PREPARATION OF FILMS FROM COLLAGEN-CONTAINING RAW MATERIALS AS INFLUENCED WITH SOME TECHNOLOGICAL FACTORS

At the previous Meetings (16th, 17th, 18th) there were presented reports on the research at the Institute on alkali-salt treatment and solubilization of certain kinds of connective tissue, as well as on the amino acid composition of the initial raw material, of collagen solubilization products (C.S.P.) and of the film materials produced therefrom /1-3/.

The degree of solubilization and the properties of the resulting C.S.P. depend on the conditions of collagen alkali-salt treatment, on the nature of the solvent and on solubilization conditions.

The present paper reports data on C.S.P. production from cattle head hides and Achilles tendons as related to alkali-salt treatment time, acid neutralization process, as well as data on the kinetics of collagen losses and non-collagenous proteins removal during preparation of the initial raw material for solubilization.

Hydrated and washed raw material was cut into 1x1 cm pieces and treated with alkali and salt during 24, 36, 48, 64 and 72 hrs under the conditions mentioned in literature previously /1/. Treatment for less than 24 hrs does not allow to solubilize the material completely. Treatment for more than 72 hrs yields C.S.P. of a lower film-forming ability.

Alkali hydrolytic activity to collagen is considerably prevented by salt. Sodium hydroxide causes significant changes in protein structure, which depend on treatment duration. The changes are revealed by means of electron microscopy and viscosimetry.

An electron microphotograph (Fig. 1) of the initial collagen shows clearly the cross-striation of the fibrils typical of the native collagen. Preparations made by means of negative contrast also show longitudinal separation of collagen fibrils into thin filaments - protofibrils. A filamentous nature of the fibrils is inherent with zones A, whereas in denser zones B it is, as a rule-

impossible to see them. The protofibrils are not strictly parallel to fibril axes and to each other and are as if bound with "hoops" (zones B). In the process of alkali-salt treatment collagen fibrils are completely disaggregated.

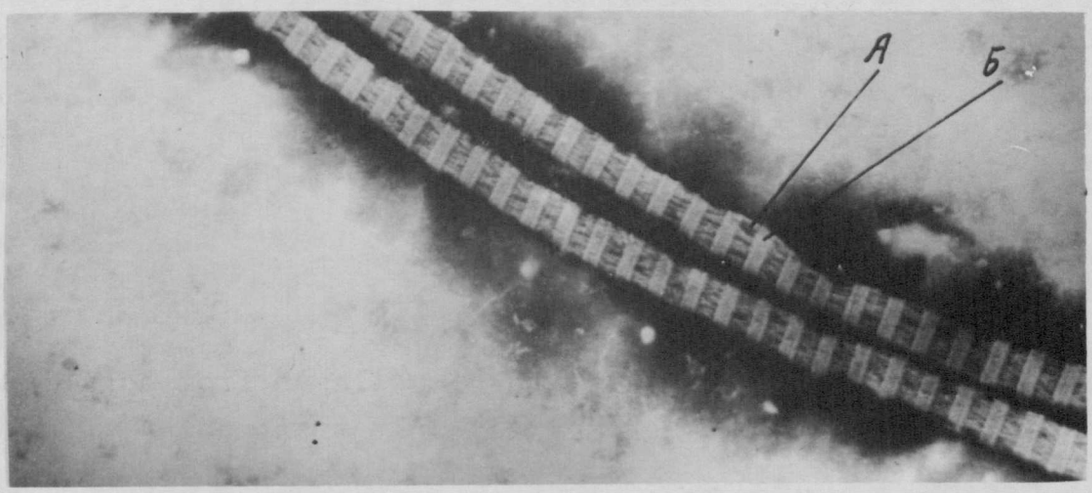

Fig. 1. Electron microphotograph of collagen from cattle Achilles tendons

During protein treatment with an alkali-salt solution for 24 hrs collagen fibrillar structure is greatly loosened, this being determined with the solubilization of the main mass of cementing substances and with the breakage of intra- and intermolecular cross-links of a covalent type, which provided the lateral association of protofibrils /4/. The effect of alkali-salt treatment in electron microphotographs is demonstrated by complete disappearance of the "hoops" fixing the protofibrils and by changing the cross-striated fibrils into homogeneous structures (Fig.2).

Treatment of collagen-containing materials with alkali and salt eliminates concomitant non-collagenous proteins, fat and, partially, carbohydrates.

The Table gives proteins loss as % of their total content during treatment of tendons and head hides.

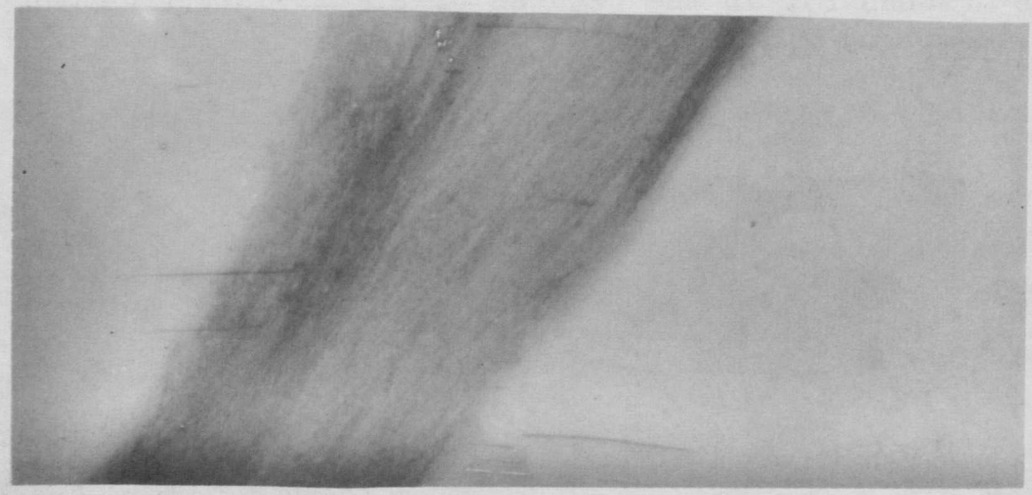

Fig. 2. Electron microphotograph of collagen from Achilles tendons after a 24 hr alkali-salt treatment

Table

Treatment	Index	Treatment time, hr.				
		24	36	48	64	72
	Protein loss during tendons treatment					
Alkali-salt	Protein	1.93	4.17	4.68	5.09	5,27
	including collagen	0,68	0,81	1.12	1,41	1,64
Neutralization	- do -	1.91	1,21	1.07	1,18	1,29
		0.62	0,53	0,51	0,53	0.72
	Total loss					
	Protein	3.84	5,38	5,75	6,27	6.56
	including collagen	1,30	1,34	1,63	1.94	2,36
	Protein loss during treatment of head hides					
Alkali-salt	Protein	4,77	4,92	5,56	6,00	6.15
	including collagen	1,06	1.18	1,53	1,76	1.76
Neutralization	- do -	1,29	1.24	1,20	0,82	0,92
		0,80	0,75	0,69	0.55	0,72
	Total loss					
	Protein	6,03	6,18	6,76	6,82	7,08
	including collagen	1,86	1,93	2,22	2,31	2,48

As is obvious from the Table, with increased alkali-salt treatment time the amount of removed noncollagenous proteins and collagen loss are rising.

Treatment for more than 36 hrs in case of tendons and for more than 48 hrs in case of head hides does not practically influence the amount of removed noncollagenous protein.

As for neutralization, the use of sulfuric and hydrochloric acids was studied. Sodium salts of these acids are easily soluble and provide a uniform removal of alkali from protein. Studies indicated that the viscosity numbers of the C.S.P., resulted from protein neutralization with 0.1 N HCl, were higher as compared to neutralization with 0.1 N H_2SO_4 (Fig. 3). Viscosimetric data show the expediency of hydrochloric acid use for neutralization.

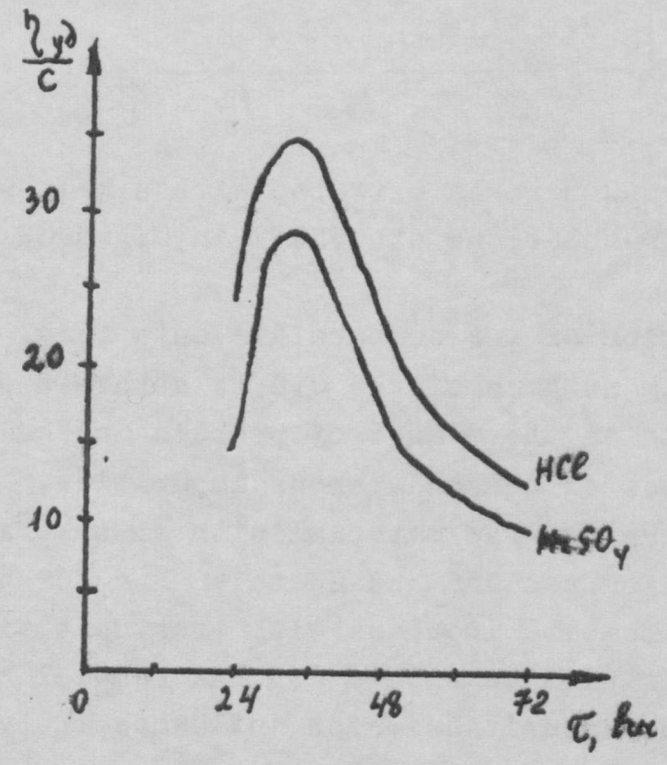

Fig. 3. Viscosity numbers as effected with neutralizing acids (0,5% solutions of C.S.P. in 0,5 N CH_3COOH)

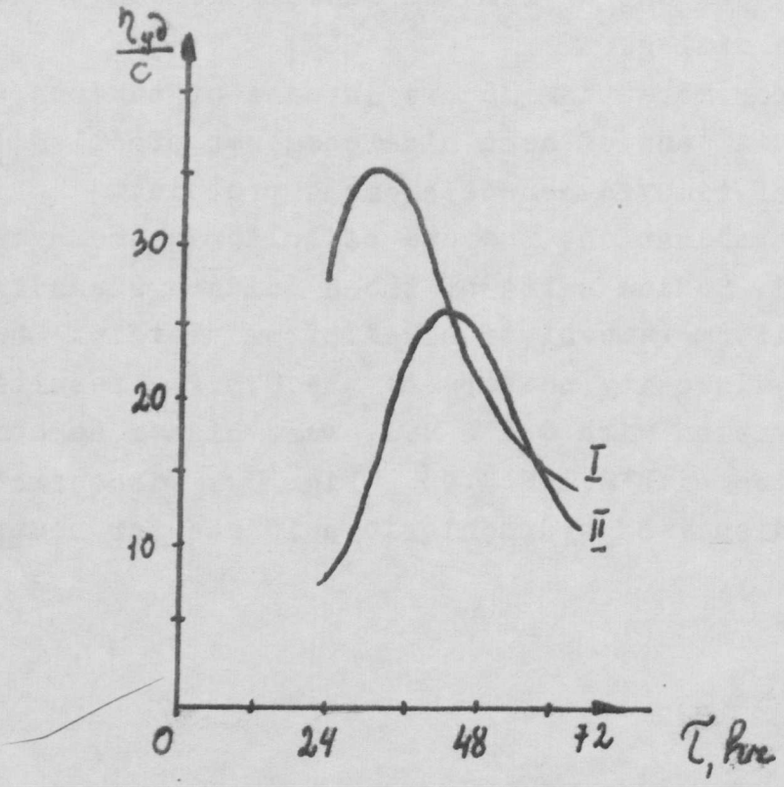

Fig. 4. Viscosity numbers as effected with alkali-salt treatment time (0,5% solutions of C.S.P. in 0,5 N CH_3COOH)

The evaluation of the comparative data (Fig. 4, Table) showed that viscosity numbers of the C.S.P. obtained depend, to a great extent, both on the degree of protein molecule destruction and on the presence of noncollagenous impurities.

When treating the raw material with an alkali-salt solution for up to 36 hrs for tendons and up to 48 hrs for head hides, C.S.P. viscosity numbers increase with treatment time. This is, probably, can be explained with a gradual removal of low-molecular noncollagenous impurities which influence the viscosity of the solutions.

A prolonged alkali-salt treatment causes a sharp decrease of solutions viscosity, this indicating, apparently, the intensification of collagen hydrolytic decomposition.

The Table shows that after 3-4-day alkali-salt treatment of the raw material losses of collagenous proteins are much faster as compared to concomitant proteins.

Inspite of "severe" conditions of alkali treatment, a three-helix structure of collagen macromolecules is retained, this being indicated by C.S.P. physico-chemical properties: a high limit viscosity number $[\eta]$ is 13-17 and specific optical rotation $[\alpha]_{546}^{20^\circ C} = -470$, which sharply fall within the temperature range 37-39°C.

The degree of particles asymmetry calculated by the limit viscosity number (Simha equation) is about 200. Sedimentation constant for the C.S.P. obtained in 0,5 N acetic acid after 34-48 hr alkali-salt treatment is S = 2.85 Svedberg units, and the molecular weight by ultracentrifugal data is $(3,2 \pm 0,7) \times 10^5$.

C.S.P., obtained according to the above technology, are, apparently, a polydisperse system composed, mainly, of three-helix rod-like particles, their molecular weight being equivalent to that of procollagen solution.

It was established that it is possible to produce fibrous and filmy structures by means of dialysis sedimentation and of additions of organic solvents, neutral salts, tanning substances, polysaccharides and by screen moulding. In the artificial materials from C.S.P., fibrillar structures with nearly regularly repeating elements are observed under an electron microscope. The cross-striation, found in reconstituted artificial fibrils, is not related to the kind of precipitators and has the reiteration period 150-300 Å (Fig. 5); in filmy materials fibrils have longitudinal filamentation (Fig. 6) similar, by its nature to the "packing" of protofibrils in the fibrils of the initial collagen after alkali-salt treatment.

The possibility to reconstitute artificial proteinaceous structures from "mature" collagen solubilization products does not only confirm the retention of the main structural units of collagen during successive alkali-salt and acid treatments, but also shows practical expediency of C.S.P. utilization to produce new filmy materials for food and medicinal purposes.

Fig. 5. Electron microphotograph of collagen fibrils obtained by C.S.P. reconstitution

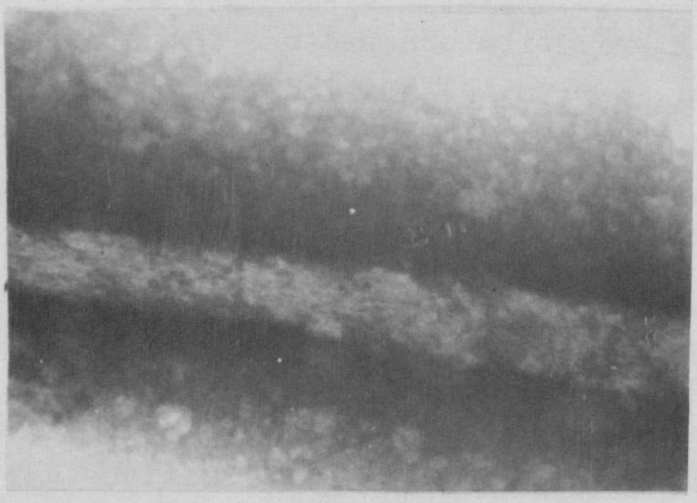

Fig. 6. Electron microphotograph of collagen fibrils in artificial filmy materials

L I T E R A T U R E

1. Балод Л.Р., Каспарьянц С.А. Исследование дермы в процессе перевода ее в растворимое состояние. XУI Европейский конгресс работников НИИ мясной промышленности, София, 1970, 914.
2. Горбатов В.М., Каспарьянц С.А., Балод Л.Р. Исследование аминокислотного состава некоторых видов соединительной ткани в процессе растворения и реконституции. XУП Европейский конгресс работниковННИИ мясной промышленности, Лондон, 1971.
3. Горбатов В.М., Каспарьянц С.А., Балод Л.Р. Изучение структурной стабильности коллагена методами инфракрасной спектроскопии и электронний микроскопии. XУШ Европейский конгресс работников НИИ мясной промышленности, Канада, 1972.
4. Баблоян О.О., Стешов Т.И., Истранов Л.П., Каспарьянц С.А., Переверзев Н.А. Электронномикроскопические исследования изменений структуры коллагена в результате щелочно-солевой обработки. "Изв.вузов", "Технология легкой промышленности", 4, 1966, 92-95.
5. Каспарьянц С.А., Баблоян О.О., Шестакова И.С. Электронномикроскопические исследования изменений коллагена в процессе растворения и реконституции, современные биохимические и морфологические проблемы соединительной ткани. Изд. "Наука", Сибирское отделение, Новосибирск, 1971, 61-64.