XXI. EUROPÄISCHER KONGRESS DER FLEISCHFORSCHUNGSINSTITUTE

ALLUNIONS-FORSCHUNGSINSTITUT DER FLEISCHINDUSTRIE DER UdSSR

STRUKTURELL-MECHANISCHE EIGENSCHAFTEN VON KOLLAGENLÖSUNGS-PRODUKTEN

W.M.Gorbatow, O.O.Bablojan, P.M.Golowanowa, G.E.Limonow, O.P.Borowikowa

Z U S A M M E N F A S S U N G

Es wurden die Kollagenlösungsprodukte untersucht, die nach Alkali-Salz-(PRK) und fermentativer Behandlung (FRK) von kollagenhaltigen Rohstoffen der Fleischindustrie (Sehnen) gewonnen wurden.

Es wurde die Abhängigkeit zwischen der Viskosität von PRK und FRK und der Eiweißkonzentration, der Veränderung von pH des Mediums sowie der mechanischen Einwirkung festgestellt.

- Die pH-Änderung bei FRK von 3,10 bis 8,60 ruft die Viskositätsabnahme um fast das zweifache hervor, während die gleiche pH-Änderung bei PRK zur unwesentlichen Senkung des Ausgangswertes führt.
- Bei den 30 Minuten bei 40°C denaturierten und nachfolgend abgekühlten PRK und FRK sinkt die Viskosität im saueren pH-Gebiet fast bis zur Viskosität des Lösungsmittels und bleibt bei der weiteren Einwirkung auf das System von Scherspannungen praktisch ohne Veränderungen.
- Im alkalischen pH-Gebiet übertrifft die Viskosität des FRK-Systems 1,5 Stunden und des PRK-Systems 20 Stunden nach der Wärmedenaturierung und nachfolgender Abkühlung die Viskosität der Ausgangsprodukte der Lösung, was mit der Bildung einer vollkommeneren Struktur aus Denaturierungsprodukten wahrscheinlich verbunden ist.

2.

Es wurde der Einfluß der mechanischen Einwirkung auf die Viskosität von PRK und FRK bei unterschiedlicher Konzentration von Lösungsprodukten untersucht. Es wurde festgestellt, daß bei der Konzentration bis 1% die Viskosität von PRK und FRK stark abnimmt, während sie bei der Erhöhung der Konzentration bis 1,5% weniger stark sinkt. Wahrscheinlicht ist es mit einem besser entwickelten Strukturnetz von konzentrierten Produkten verbunden.

Die erhaltenen Ergebnisse können bei der Herstellung von künstlichen Kollagenmaterialien (Wursthüllen, Fleischwarenüberzügen, künstlichen Kollagenfaden u.a.m.) ausgenutzt werden.

XXIst EUROPEAN MEETING OF MEAT RESEARCH WORKERS

ALL-UNION RESEARCH INSTITUTE OF THE MEAT INDUSTRY

STRUCTURAL-AND-MECHANICAL PROPERTIES OF COLLAGEN SOLUBILIZATION PRODUCTS

V.M.Gorbatov, O.O.Babloyan, P.M.Golovanova, G.E.Limonov, O.P.Borovikova

S U M M A R Y

Collagen solubilization products, resulting from alkali-salt (PRC) and enzymatic (FRC) treatments of collagen-containing wastes of meat production (tendons), have been studied.

Relations of PRC and FRC viscosity to protein content, medium pH and mechanical treatment have been established:

- a change in FRC pH from 3.10 up to 8.60 reduces the viscosity by nearly two times, whereas a similar change in the pH of PRC decreases the initial viscosity only slightly;
- in the acid range, the viscosity of PRC and FRC following denaturation at 40°C for 30 min. and cooling, decreases down almost to the viscosity of the solvent and further, practically, does not change under shear stresses;
- in the alkaline range, after heat denaturation and cooling, the viscosity of FRC in 1.5 hr and that of PRC in 20 hr exceeds the viscosity of the initial solubilization products, this, obviously, being due to the formation of a more perfect structure from denaturation products.

The effect of mechanical treatment upon PRC and FRC viscosity at various levels of solubilization products was also studied. It was found that PRC and FRC viscosity at the concentration of up to 1% decreased sharply under mechanical treatment,

2.

while at 1.5% this decrease was less intensive, this being, probably, connected with a more developed structural network in concentrated products.

The results obtained can be used in the production of artificial collagenous materials (sausage casings, coatings for meat products, man-made collagenous threads, etc.).

XXI ЕВРОПЕЙСКИЙ КОНГРЕСС
РАБОТНИКОВ НИИ МЯСНОЙ ПРОМЫШЛЕННОСТИ

Всесоюзный научно-исследовательский институт
мясной промышленности СССР

СТРУКТУРНО-МЕХАНИЧЕСКИЕ СВОЙСТВА ПРОДУКТОВ РАСТВОРЕНИЯ КОЛЛАГЕНА

В.М.Горбатов, О.О.Баблоян, П.М.Голованова, Г.Е.Лимонов,
О.П.Боровикова

А Н Н О Т А Ц И Я

Исследованы продукты растворения коллагена, полученные после щелочно-солевой (ПРК) и ферментативной (ФРК) обработок коллагенсодержащих отходов мясной промышленности (сухожилий).

Установлена зависимость, связывающая вязкость ПРК и ФРК с концентрацией белка, изменением pH среды, механическим воздействием:

- изменение pH ФРК с 3, I0 до 8,60 вызывает уменьшение вязкости почти в два раза, тогда как такое же изменение pH у ПРК приводит к незначительному снижению исходного показателя;
- в ПРК и ФРК в кислой области pH, подвергнутых денатурации при 40°С в течение 30 мин. с последующим охлаждением, вязкость снижается почти до вязкости растворителя и в дальнейшем практически не изменяется при воздействии на систему сдвиговых напряжений;
- в щелочной области pH после тепловой денатурации и последующего охлаждения, вязкость системы для ФРК через I,5 часа, и для ПРК через 20 час. превышает вязкость исходных продуктов растворения, что, по-видимому, связано с образованием более совершенной структуры из продуктов денатурации.

Исследовано влияние механического воздействия на вязкость ПРК и ФРК при различных концентрациях продуктов растворения, так при концентрации до I% она резко уменьшается, а при увеличении концентрации до I,5% - в меньшей степени, что, вероятно, связано с более развитой структурной сеткой в концентрированных продуктах.

Полученные результаты могут быть использованы в производстве искусственных коллагеновых материалов (колбасных оболочек, покрытий на мясопродукты, искусственных коллагеновых нитей и др.).

XXIst EUROPEAN MEETING OF MEAT RESEARCH WORKERS

ALL-UNION RESEARCH INSTITUTE OF THE MEAT INDUSTRY

STRUCTURAL-AND-MECHANICAL PROPERTIES OF COLLAGEN SOLUBILIZATION PRODUCTS

V.M.Gorbatov, O.O.Babloyan, P.M.Golovanova, G.E.Limonov,
O.P.Borovikova

In the recent years a great number of papers appeared (1-5) on the use of collagen-containing materials in the production of artificial sausage casing, coatings for meat products, etc.

Collagen is most available of all the animal proteins. A growing interest in collagen is due to the fact that it can be relatively easily isolated in a pure form as soluble macromolecules and is of great chemical and thermal stability, as well as of a unique, highly ordered structure. Fibrous collagens can be treated chemically, mechanically, etc. Resulting solubilization products possess the properties close to those of native collagen solutions (pro-collagen) and retain the natural three-helical structure of macromolecules.

Peculiarities of diluted collagen solutions have been studied in detail (2), while the properties of concentrated solutions are studied very little despite the fact that recently they have found commercial application.

This paper reports the results of a study into the rheological properties of collagen solubilization products, which determine the behaviour of the latter during the formation of film coatings, oriented fibers, etc. These data are of considerable interest for elucidating the conditions under which collagen solubilization products can be used as film-formers.

It is known (6, 7) that in disperse systems and solutions of high molecular compounds, there is a spatial structural network which constitutes a loose carcass made of primary particles, of their chains and aggregations resulting from intermolecular interactions of structural elements with one another and with solvent molecules. Structuro-mechanical properties of a system are determined with the strength of the spatial structural network. The nature of deformation processes during flow of disperse or high-molecular systems is determined with relaxation phenomena of destruction and reconstitution of the structures existing in the system, the former being dependent upon the peculiarities in the structure of polymer macromolecules.

Collagen solubilization products are structured liquids and characterized with a relation of effective viscosity to the operating stress. In such liquids, every rate of deformation in a stationary flow has a corresponding degree of structure equilibrium breakage.

Experimental

Objects of the study were collagen solubilization products derived by means of an alkali-salt method (PRC) (1, 2), and products of enzymically solved collagen (FRC) obtained according to the VNIIMP method of collagen (beef Achilles tendons) digestion with pepsin.

The viscous properties of these products were studied at protein concentrations from 0.8 up to 2.0%. For the experiments, a viscosimeter "Rheotest" Type RV (GDR) was used, which allows to measure viscosity within a broad range of shear rates. To derive flowability curves, shear rates were changed stepwise from 0.3 up to 1.312 sec^{-1}. Preliminary tests indicated that, in case of shear deformation of collagen solubilization products, the equilibrium between the structure being destroyed and that

being reconstituted was recorded by the end of the first minute of deformation. Therefore, in the experiments the deformation for any given shear velocity was continued for 1 minute.

The experimental data obtained allowed to find a relation of the effective viscosity of collagen solubilization products of different concentrations to a shear rate (Fig. 1). This relation can be described with the following equation (8):

$$\eta_{ef.} = B^{*} \cdot \dot{\varepsilon}^{-m},$$

where B^{*} - effective viscosity at $\dot{\varepsilon} = 1$ sec^{-1};
$\dot{\varepsilon}$ - deformation rate, $sec.^{-1}$;
m - rate of structure breakage, or flow index.

From Fig. 1 it is clear, that effective viscosity relations to shear rates within the forementioned range of rates in logarithmic co-ordinates are, practically, parallel straight lines. Thus, flow index is a constant for any type of solutions, for FRC it being 0.775 and for PRC 0.860. Therefore, we can compare the effective viscosity of the test solutions at the same deformation rate.

Fig. 2 shows changes in FRC and PRC effective viscosity as related to concentration. It is clear that for FRC this relation is less vivid than for PRC. E.g., at 1.5% concentration FRC viscosity is 3 times as low as that of PRC, this, possibly, being due to differences in the aggregation sizes of collagen solubilization products obtained by the two methods.

The viscosity of the solutions remains high, this preventing the preparation of equally dense films and fibrous materials. To decrease PRC viscosity, the authors studied the effect of pH, temperature and mechanical treatment. pH of FRC was changed from 3.10 up to 8.60 and that of PRC from 3.10 up to 8.30 with NH_4OH solution. Figs. 3 and 4 demonstrate FRC viscosity falling nearly by twice and PRC viscosity changing only slightly. This, obviously, points to a higher deformability of

the collagen molecular rods of FRC and of their aggregations with changing pH of the medium.

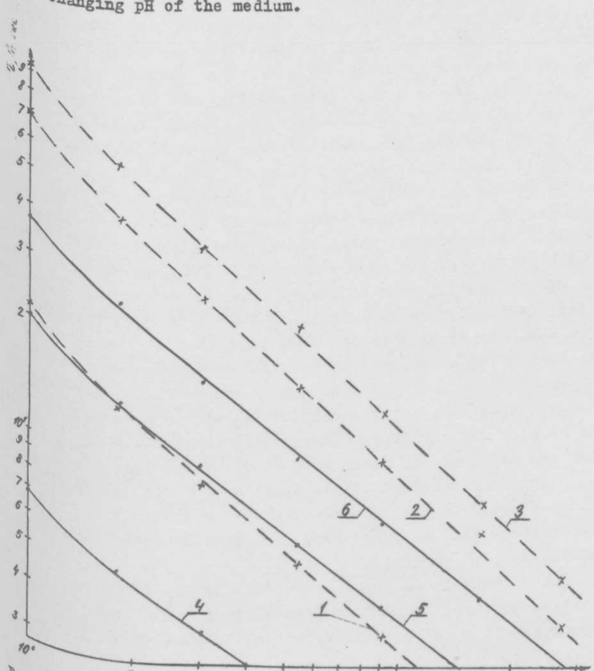

Fig. 1. Changes in PRC and FRC viscosity as related to deformation rate at pH=3.10 and t=20°C
- - - - PRC concentration, %; 1 - 1.00, 2 - 1.55; 3 -1.75
——— FRC concentration,%; 4 - 1.00, 5 - 1.50, 6 -2.15

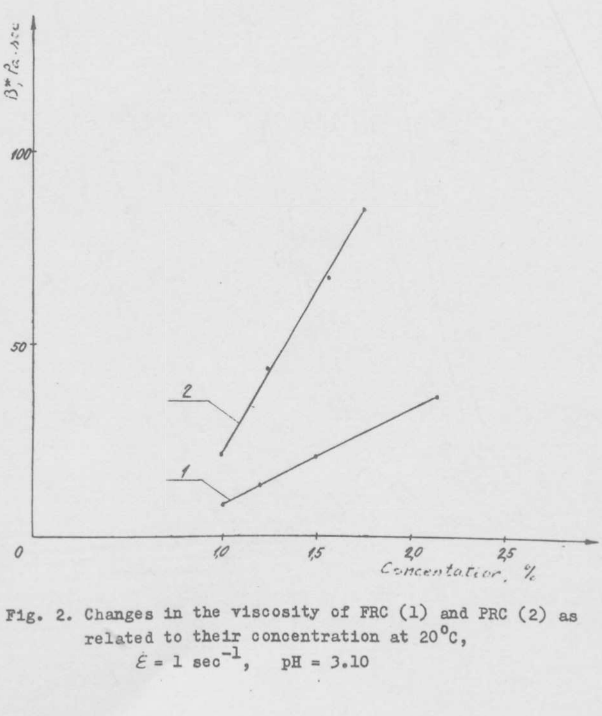

Fig. 2. Changes in the viscosity of FRC (1) and PRC (2) as related to their concentration at 20°C, $\dot{\varepsilon} = 1$ sec^{-1}, pH = 3.10

6.

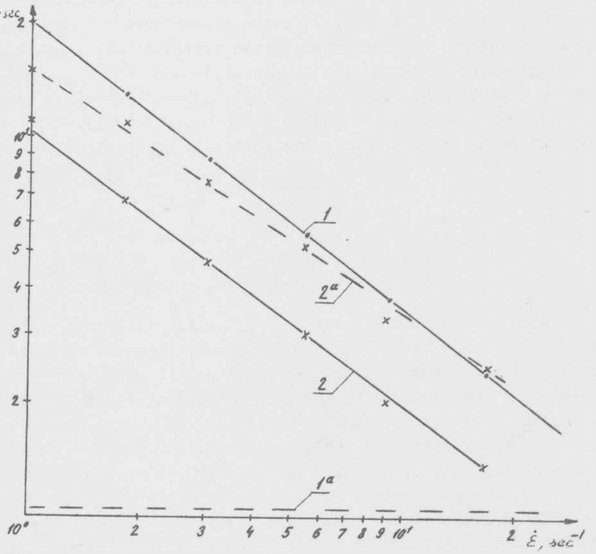

Fig. 3. Changes in viscosity as related to the deformation rate of the initial and denaturated 1% FRC at acidic and alkaline pH's at 20°C

1 - initial FRC - pH = 3.10

1^a - denaturated FRC - pH = 3.10

2 - initial FRC

2^a - denaturated FRC and renaturated for 1.0; 1.5; 3.0; 5.0 and 20 hrs

7.

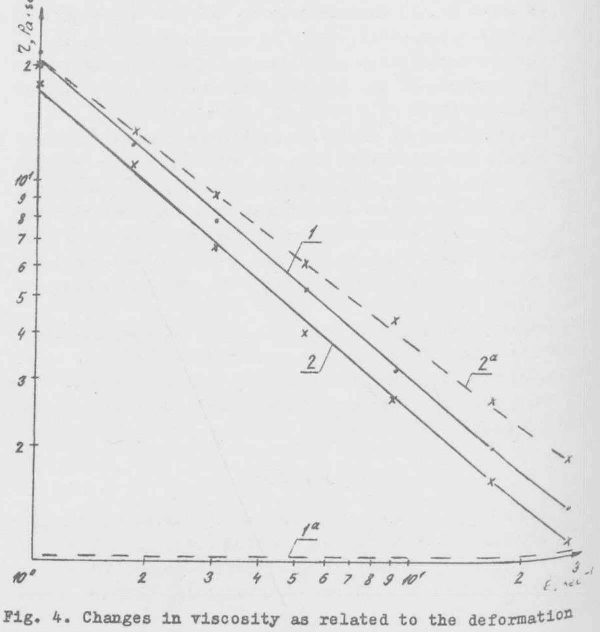

Fig. 4. Changes in viscosity as related to the deformation rate of the initial and denaturated 1% PRC at acidic and alkaline pH at 20°C

1 - initial PRC - pH = 3.10

1^a- denaturated PRC - pH = 3.10

2 - initial PRC - pH = 8.30

2^a- denaturated PRC and renaturated for 20 hrs - pH = 8.30

8.

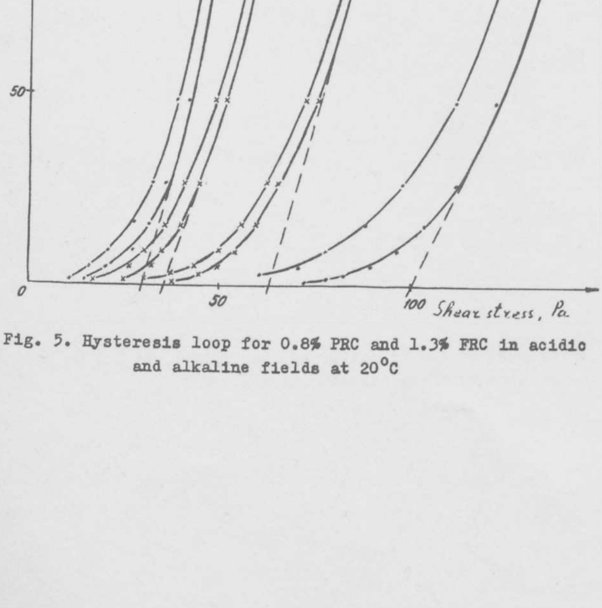

Fig. 5. Hysteresis loop for 0.8% PRC and 1.3% FRC in acidic and alkaline fields at 20°C

9.

Acidic and alkaline PRC and FRC were heated in a thermostat at 40°C for 30 min. for "mild" heat denaturation to occur with the destruction of the links stabilizing the native structure of collagen. After heating, denaturated PRC and FRC were cooled in a thermostat for 30-40 min. down to 20°C. The viscosity was measured just after heating and after cooling with the intervals of 1; 1.5; 3; 5 and 20 hrs. In acidic denaturated FRC and PRC, the viscosity was noted to decrease nearly down to that of the solvent (Curves 1a in Figs. 3 and 4)and then to remain unaltered under shear stresses. In alkaline denaturated FRC and PRC just after heating the viscosity fell sharply, but afterwards it increased significantly (for 1.5 hr in case of FRC and for 20 hrs in case of PRC), somewhat exceeded that of the initial solubilization products and became stable at this level (Curves 2a in Figs. 3 and 4). This process is, evidently, connected with the formation of a more perfect structure during the renaturation of "mild"-denaturation products.

From the initial and denaturated acidic and alkaline products of collagen solubilization, films were cast on a polyethylene backing and air-dried. The welding temperature of these films was measured, it being 43-47°C for the films from the initial acidic and alkaline solubilization products and 30-31°C for the films from denaturated alkaline products. Films from denaturated acidic products were dissolved in water during swelling without heating.

These results, as well as the data on viscosity, prove a significant effect of pH upon denaturation temperature and structure-mechanical properties of denaturated solubilization products.

For structured systems, with collagen solubilization products belonging to them, it was of interest to follow changes in the relative ultimate shear with pH (Fig. 5). For FRC with pH=3.10, this value, characterizing structure strength, is equal to 100 Pa and for FRC with pH=8.60 it equals 30 Pa, i.e.

10.

in the acidic field is more than 3 times as high. This proves higher strength of the structural network of FRC in the acidic medium. As for PRC, this phenomenon is also observed but is less pronounced (at pH=3.10 this value is equal to 63 Pa and at pH=8.30 - to 37 Pa).

The effect of shear stresses was studied on 1.3% FRC at pH=3.10 and 8.60 and on 0.8% PRC at pH=3.10 and 8.30 as with higher concentrations the internal cylinder of the viscosimeter slipped relative to the test solution at great deformation rates.

The effect of shift stresses upon FRC at pH=3.10 was found to result in a sharp change of the viscosity and well-pronounced hysteresis, this indicating the breakage of the structural network. In the alkaline field these effects were smaller for FRC due to the development of a stronger structure. In case of PRC both in the acidic and alkaline fields, hysteresis was less pronounced.

It is known (8) that mechanical treatment is of a considerable effect upon the viscosity of structured systems. With this in view, we investigated a similar effect upon PRC and FRC at various concentrations and at a similar pH value, viz., 3.10. Mechanical mixing was performed in a laboratory homogenizer for 10 min. at 1,000 rpm. The data in the Table show that solution viscosity rises greatly with PRC and FRC concentration increasing up to 1.5%, this being due to structure strengthening.

Hydrated protein systems have a structural network which is not only very strong, but elastic and resilient, this allowing the above systems to damp the stresses from outside. Therefore, a mechanically destroyed hydrated protein system is able to recombine comparatively fast, this resulting in a new and sometimes, a stronger structure.

11.

T a b l e

Changes in PRC and FRC viscosity at the deformation rate ($\dot{\varepsilon}$) of 1 sec.$^{-1}$

Product	Concentration, %	Viscosity of the initial solution, Pa·sec.	Viscosity after mixing, Pa·sec.	Decrease in viscosity, %
FRC	0.8	13.60	3.93	71
PRC	0.8	26.90	9.40	65
FRC	1.5	45.50	36.00	21
PRC	1.5	112.00	103.5	7.5

Obviously, under the mechanical destruction of collagen solubilization products of a higher concentration, structure reconstitution is more vivid; post shear stresses the viscosity of concentrated systems during mixing decreases insignificantly. In systems of a low protein concentration, mechanical treatment causes great destruction of a poorly-developed structure and reduces its viscosity by nearly three times.

Conclusions

The results obtained allowed to draw the following conclusions:

1. Viscosity relation to PRC or FRC concentration in the range tested (0.8 to 2%) is of a linear character. FRC and PRC viscosity rises with protein level, this, probably, being due to the development of a stronger structural "carcass" in concentrated systems.

2. Changes in FRC pH from 3.10 up to 8.60 decrease the viscosity of the system by nearly two times, while similar changes in PRC pH cause only a slight fall in the initial value. Shear stresses alter sharply the viscosity of FRC having the pH of 3.10 with well-pronounced hysteresis. At pH = 8.60 for FRC and at pH either 3.10 or 8.30 for PRC, hysteresis is

12.

much less pronounced.

3. Heat denaturation of PRC and FRC solutions with pH = 3.10 causes irreversible changes in the system, whereas a similar treatment at pH = 8.30 or 8.60 somewhat increases the viscosity of renaturated products as compared to the initial one due to the formation of a more perfect structure from the products of "mild" denaturation.

4. Mechanical treatments destroy considerably weakly-developed systems with a low protein concentration (up to 0.8%); similar treatments of more concentrated systems (up to 1.5%) have no considerable influence upon the viscosity of these systems due to their high strength and elastic and resilient properties.

Literature

1. М и н к и н Е.В., Ш е с т а к о в а И.С. Способ растворения шкур животных. Авт. свид. СССР № I62280 кл. 286 от 26.07.I962.
2. Г о р б а т о в В.М., Б а б л о я н О.О., Б а л о д Л.Р., К а с п а р ь я н ц С.А. Влияние некоторых технологических факторов на получение пленочных материалов из коллагенсодержащего сырья. XIX Европ. конгр. работн. НИИ мясн. пром-сти, Париж, 3, I973, I3I5.
3. Патент ФРГ "Способ производства растворимых коллагенов". № I288237 выдан 30.0I.I969.
4. Патент США "Коллагеновый материал и способ его получения" № 3II4372 кл. I28-335.5 выдан I7.I2.I963.
5. Патент Японии "Способ растворения коллагенового волокна под действием кислой протеазы" № II037 выдан 30.0I.I969.
6. М и х а й л о в Н.В., Р е б и н д е р П.А. О структурно-механических свойствах дисперсных и высокомолекулярных систем. "Коллоид. журнал", I7, 2, I955, I08.
7. Р е б и н д е р П.А. Физико-химическая механика. Серия IУ, 39-40, М., изд-во "Знание", I958.

I3.

8. Р о г о в И.А., Г о р б а т о в А.В. Новые физические методы обработки мясопродуктов. М., изд-во "Пищевая промышленность", I966.