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جلد ۱۱، شماره ۳، مرداد - شهریور ۱۳۹۴، ص. ۲۸۴-۲۷۹

Short Communication

Microstructural changes in condensed milk with the starch syrup during prolonged storage: an electron microscopy study

Igor T. Smykov[1]*, Anna I. Gnezdilova[2], Yuliya V. Vinogradova[2], Valentina L. Popova[2]

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Abstract

These work deals with the study of changes in the microstructure of the sweetened condensed milk with sugar substitution for starch syrup during its long storage period. The research has been carried out with the transmission electron microscope. The authors have analyzed the microstructure of the condensed milk with sugar and the microstructure of the condensed milk with 40% and 100% sugar substitution for the starch syrup. It is established that filamentous bridges are formed between the casein micelles in the microstructure of the condensed milk with the partial sugar substitution for the starch syrup. These bridges are pseudo-polymers, formed by glucose monomers. During the long-storage period the number and density of these bridges increase, influencing, consequently, the organoleptic and rheological properties of the condensed milk. The studies have shown that there is a critical concentration of the starch syrup in the condensed milk, which limits the syrup use in the manufacture of the product.

Keywords: Condensed milk, Starch syrup, Microstructure, Electron microscopy, Pseudo polymer.

Introduction

Sweetened condensed milk is one of the most common long-life dairy products. Its rheological parameters have undergone significant changes over time and depend on the storage conditions, the composition and the product processing techniques.

Sweetened condensed milk is, in its essence, a gel characterized by a specific structure, which determines its rheological properties. Therefore, there are many experimental works dedicated to the condensed milk structure analysis that use different research methods. Electron microscopy methods are widely used in condensed milk structural feature studies. There are many publications in the scientific literature that describe various methods of specimen preparation, preparation equipment and implementation of various electron microscopy research methods. In each specific instance, the method of specimen preparation for a research is chosen on the basis of the electron microscope capabilities, the research objectives and the research object characteristics.

Source (1) studies the effect of polyphosphates on the gelation process in the concentrated skim milk when it is stored for a long period. A transmission electron microscope has been used in the study. The test concentrated milk sample has been fixed with glutaraldehyde, and microscopy agent staining has been carried out by sputtering a thin layer of platinum on the vacuum surface. The studies have revealed the effect of the introduced polyphosphates on the size and structure of the casein micelles and on the structure of the gel formed by them. There is also a possible explanation of the occurring gelation process slowdown.

Source (2) is aimed at the study of changes in rheological properties and the microstructure of the sweetened condensed milk samples in their long-term storage, as well as at the effect of various additives on

1. professor Department of physical chemistry, All-Russian Research Institute of Butter and Cheese Making, Uglich, Russia
2. Associate professor Department of technological equipment, Vereschagin State Dairy Farming Academy, Vologda, Russia
(*- Corresponding Author Email: i_smykov@mail.ru)

these changes. Transmission electron microscopy has been used in these studies; the specimen preparation has been carried out by the ultramicrotome method with the primary glutaraldehyde sample fixation and with the subsequent osmium tetroxide fixation. The analysis of the images shows that the aggregation of the micelles and the casein micelle association with fat globules increase during the storage period. Whey proteins play an important role in the condensed milk viscosity growth.

A transmission electron microscope has been also used in source (3), but the specimen preparation has been carried out by the ultramicrotome method with fixing samples with osmium tetroxide only. This method has allowed the authors to identify the nature of the protein particle interaction with themselves and with milk fat globules. It is shown that the condensed milk microstructure depends primarily on the temperature and concentration of the solid components.

In source (4), the study of the concentrated skim milk structure has been carried out with the help of the transmission electron microscope by implementing three different methods of specimen preparation. In the first case, the specimen study has been carried out directly with its preliminary glutaraldehyde fixation. This method has allowed to determine the size and shape of the casein micelles. However, the micelle surface microstructure determination has turned out to be impossible by the use of this method. In the second case of the specimen preparation the freeze-cleave method – the cryoprotected sample replication has been used. Glycerol has been used as the cryoprotectant. The resulting images have revealed the internal structure of the casein micelles. Since cryoprotection comprises dissolving the sample with glycerin, the interaction of micelles in the concentrated milk cannot be observed. In the third case, ultra-rapid freeze-cleave-etching pure sample replication method has been used. This method has allowed determining the differences in the micelle internal microstructure under the influence of external factors. The use of these three research methods has allowed to eliminate some preparation artifacts and to get some new complementary information concerning the casein micelle structure and their aggregation in concentrated skim milk.

Nowadays, more and more attention is paid to the development of dairy products that have functional properties and that are intended for certain population groups: children, the elderly, athletes and others. In particular, the production technology of sweetened condensed milk with the sucrose substitute for the glucose syrup is under development. Moreover, the insurance of the specified rheological properties of such products during their long storage period is very important. At the same time, the condensed milk microstructure, its change during the storage period and the relationship of the microstructure and rheology remain unexplored.

The purpose of this electron microscopic study is to identify morphometric structure characters formed by the casein micelles in the sweetened condensed milk, and their interaction with the starch syrup during the long-term storage.

Material and Methods

Determination of condensed milk

The study includes six condensed milk samples with sugar and the starch syrup produced in the Vereshchagin State Dairy Farming Academy of Vologda. The moisture content of the samples are 26.5%; carbohydrates - 43.5%; dry milk solids - 28.5%, including 8.5% of fat and 34.0% of protein in nonfat milk solids. The mass fraction of sugar substitute for the glucose syrup in the samples and periods of their storage are given in Table 1.

Table 1. The proportion of sugar substitutes by the starch syrup and periods of sample storage

Sample	1	2	3	4	5	6
Replacing sugar, %	0	40	100	0	40	100
Storage periods, months	0	0	0	18	18	18

In samples 2, 3, 5, 6 the used starch syrup corresponds to the Russian state standard specification GOST 52060-2003. All samples have been stored at 20 ± 2 °C.

Determination of electron microscopy

The prepared samples have been examined in a Philips transmission electron microscope (TEM) (Philips, EM-410, MD Eindhoven, Netherlands) at an acceleration voltage of 60 kV. The specimen preparation for the analysis has been performed as follows. The dose of the test sample, that is being in the form of a colloidal solution prepared by dissolving the condensed milk in the distilled water in a ratio of 1:100, has been applied on the metal supporting grid covered with an electron-transparent polymer film. Then the specimen have been fixed with glutaraldehyde, the used method is described by (4-6). Staining with uranyl acetate has been used to identify small parts of the condensed milk structure. After fixation, staining, washing and drying, a grid with the applied specimen has been taken for the electron microscopy studies.

The image analysis has been carried out by the use of Adobe Photoshop CS (Adobe Systems Inc., San Jose, CA, USA). To assure the relevance of the segmentation procedures, every segmented micrograph has been visually compared with its original one.

Result and Discussion

The comparative electronic microscopy morphometric analysis of microstructures and characteristics of the particles formed in the condensed milk, depending on the concentration of the glucose syrup and the shelf life has been conducted during the experimental studies. The microstructure of the condensed milk samples, having different starch syrup contents, as well as the microstructure of the samples, having the same starch syrup contents, but different storage period have been compared.

Figure 1 shows electron microscopic photographs of sweetened condensed milk specimens, obtained by the instrumental magnification $14000^{\times}$. This magnification is chosen in order to assess the structural characteristics of the samples in general. On the left there are pictures of the specimen of freshly condensed milk samples, and on the right there are photos of the condensed milk specimen that have been stored for 18 months.

Comparing images of the control microstructure samples of freshly sweetened condensed milk with microstructure samples stored for 18 months received at the low magnification (Fig. 1), it can be noted that in both samples the overall microstructure is characterized by friable unbounded casein micelle aggregates. If we compare the images of the microstructure of the condensed milk samples with 40% sugar substitution for the glucose syrup of freshly condensed milk and the milk stored for 18 months between each other and the images of the microstructure of the control samples the following can be seen:

- the general view of the microstructure of the fresh sample with 40% sugar substitution for the starch syrup is fairly close to the general microstructure of the test sample after the long-term storage;
- long, well-defined pseudo polymer bridges (filaments), bounding individual aggregates of the casein micelles are formed in the microstructure of the sample with 40% sugar substitution after the long-term storage;
- casein micelle aggregates in the microstructure of the sample after the long-term storage are much denser than the aggregates in freshly condensed milk sample.

The microstructures of the fresh sample with 100% substitution of sugar for the starch syrup and the sample after a long storage period, as compared with the previous samples, are generally characterized by the blurred image of the casein micelles, which seems to be caused by the interaction of glucose molecules with the surface of the micelles. However, in the sample with 100% substitution of sugar after a long storage period, the casein micelles have a sharper image and their visible sizes are smaller than the original ones.

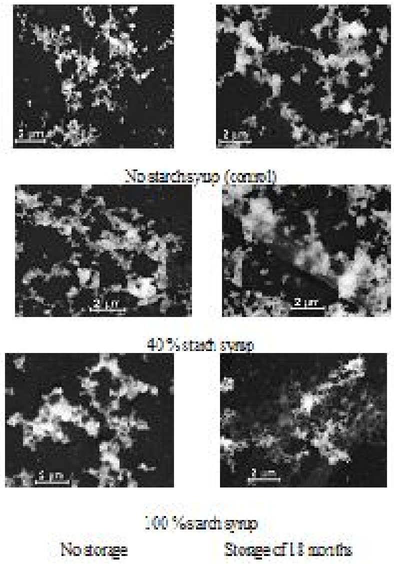

Fig. 1. Effect of the starch syrup, and the total storage time of the sweetened condensed milk microstructure.

t the same time the number of filamentous bridges have increased and they have become sharper. These changes in the microstructure during the storage period are probably caused by the glucose monomer pseudo polymerization processes.

Photographs of these condensed milk specimens produced at a higher magnification tool (52000$^{\times}$) are shown in Figure 2. This magnification (Fig. 2) shows that there are filamentous bridges between the casein micelles in the microstructure of the fresh sample, as well as in the microstructure of the control sample after the long-term storage, and in the sample with 40% sugar substitution for the starch syrup after a long storage period, such bridges become wider and sealed. This means that glucose is involved in the formation of the filaments. It is also obvious that such a significant change in the microstructure of the sample after storage must be accompanied by a viscosity growth, compared to the fresh sample. The mechanism of formation of such additional bounds between the casein micelles during a long-term storage requires special investigation and explanation.

The microstructures of the samples of the freshly condensed milk with 100% sugar substitution for the starch syrup and condensed milk that have been stored for 18 months, also markedly different from each other and from the microstructures of the samples examined above. Primarily these differences consist in the following: as for the long-term storage sample, a connected structure, in which it is difficult to distinguish individual aggregates of micelles, is formed there, where the main structural element is not casein micelles, but the starch syrup, which forms a spatial structure with branched (pseudo-) polymerization of monomers of its components. When comparing the images of the microstructure obtained at higher magnification (Fig. 2), it is clearly visible that the long-term storage is accompanied by sealing filamentous bridges that link the casein micelles. Such a seal can be explained by dehydration of the filament structure of the starch syrup.

For a clearer view of the nanostructure formed while storing the condensed milk its image with magnification of 112000$^{\times}$ has been made (Fig. 3). (Pseudo) polymeric filamentous bridges between casein micelles promote the formation of the single spatial structure.

If filamentous bridges have pseudo polymer nature, i.e. non-covalent bond between the monomers, the viscosity and thixotropic of the condensed milk with 100% sugar substitution for the glucose syrup, after a long-term storage should increase dramatically. If the viscosity increases and almost no thixotropic are observed, these bridges have purely filamentous polymeric nature.

Glucose as the main component of the starch syrup can, over time, form pseudo polymer and its monomeric units are joined with non-covalent - by hydrogen and hydrophobic bonds. This phenomenon may play a significant role in forming pseudo structures during the condensed milk storage. The formation of pseudo polymer structures have been recently paid much attention. For example, source (7) deals with the construction of supramolecular structures on the basis of pseudo polymers that are formed by hydrogen bonds. Source (8) gives the analysis of the pseudo-polymerization effect on the visco-elastic properties of hydrogels. Thesis (9) studies the interaction of proteins and polysaccharides and the formation of these pseudo polymer structures.

Thus, there is every reason to suppose that during the long-term storage of the condensed milk with sugar substitution for the starch syrup pseudo-polymer structures affecting the organoleptic and rheological properties of the product can be formed.

Conclusion

The article shows that filamentous bridges are formed between casein micelles in the microstructure of the condensed milk with sugar substitution for the starch syrup after a long-term storage. These bridges probably represent pseudo-polymers formed by glucose monomers, and determine the microstructure of the product, its organoleptic and rheological properties.

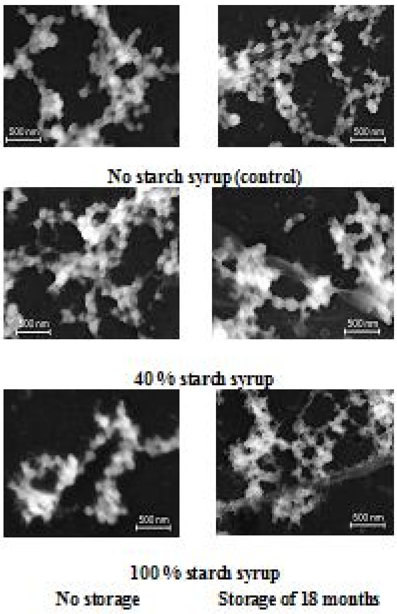

Fig. 2. Effect of the starch syrup content and storage time on the nanostructure of sweetened condensed milk.

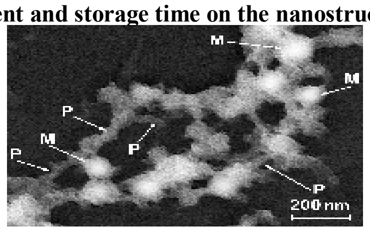

Fig. 3. Formation of nanostructures in condensed milk with the addition of the glucose syrup (100%); M - casein micelles, P - (pseudo) polymer bridges.

The mechanism of formation of additional bonds between the casein micelles during a long-term storage requires special investigation and explanation. The studies have shown that there may be a of the starch syrup critical concentration in the condensed milk, limiting the starch syrup application in the product manufacture. However, the glucose syrup may have different saccharide compositions and therefore different glucose contents. Thus, the starch syrup critical concentration in condensed milk is determined by its composition.

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مقاله کوتاه پژوهشی

تغییرات ریزساختاری در شیر تغلیظ شده با شربت نشاسته در طول ذخیره سازی طولانی مدت : یک مطالعه میکروسکوپ الکترونی

Igor T. Smykov[1]*, Anna I. Gnezdilova[2], Yuliya V. Vinogradova[2], Valentina L. Popova[2]

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چکیده

مطالعه تغییرات ریزساختار شیر تغلیظ شده شیرین با قند، جهت جایگزینی شربت نشاسته در طول دوره ذخیره‌سازی طولانی مدت مورد بررسی قرار گرفت. این پژوهش با میکروسکوپ الکترونی عبوری انجام شد. نویسندگان، ریزساختار شیر تغلیظ شده با قند و ریزساختار شیر تغلیظ شده با ۴۰٪ و ۱۰۰٪ شربت نشاسته را مورد تجزیه و تحلیل قرار دادند. نتایج نشان داد که پل رشته‌ای میان میسل کازئین در ریزساختار شیر تغلیظ شده با جایگزینی قند جزئی تشکیل شد که این پل‌ها شبه‌پلیمر هستند و با منومرهای گلولز تشکیل شده‌اند. در طی دوره ذخیره‌سازی بلند مدت، تعداد و چگالی این پل‌ها افزایش یافت که بالتبع موثر بر خصوصیات حسی و رئولوژیکی شیر تغلیظ شده بود. این مطالعه غلظت بحرانی شربت نشاسته را در شیر تغلیظ شده و محدودیت استفاده از آن را در تولید کالا نشان داد.

واژه‌های کلیدی: شیر تغلیظ شده، شربت نشاسته، ریزساختار، میکروسکوپ الکترونی، شبه‌پلیمر.

1. professor Department of physical chemistry, All-Russian Research Institute of Butter and Cheese Making, Uglich, Russia
2. Associate professor Department of technological equipment, Vereschagin State Dairy Farming Academy, Vologda, Russia
(*- Corresponding Author Email: i_smykov@mail.ru