# PRODUCTION OF POLYELECTRLYTE NANOMICROCAPSULES FOR IMMOBILIZATION OF FOOD INGREDIENTS

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***Abstract*:. Production of micronanoemulsions of biologically active substances, being used for production of meat-based preventive-curative products is described. It is shown that using the immobilization of labile vitamins and amino acids in water-fat emulsions with stabilizers on the basis of natural polyelectrolytes - sodium alginate, gums, carragheenan and carboxymethylcellulose - it is possible to obtain emulsified systems, possessing increased stability, for example, to thermal regimes, used in production of meat products.**

***Index Terms*: - amino acids, functional products, micronanoemulsion, vitamins**

## 1. INTRODUCTION

Micronanoencapsulation can be considered as a possible approach to change in lability of biologically active substances (BAS) for food and pharmaceutical applications. As is known, vitamins and some amino acids are sufficiently labile BAS. The efficiency of their use is limited due to thermal regimes of sterilization in industrial production of meat products. Thus, the common steam sterilization at $121^0$C during 1 hour leads to destruction of many BAS, vitamins included. Inclusion of labile vitamins into protective micro- and nanocapsules can change temperature stability of these substances.

Micronanoencapsulation is an inclusion of BAS into particles, nominal size of which can be from several μm (microlevel) to nm (nanolevel). Such inclusion is essentially the generation of small micro- or nanoparticles, which due to polyelectrolyte interactions in multicomponent solution became covered with protective coatings from other natural biopolymers (Nekludov, Ivankin, 2003).

This work describes obtaining of micronanoemulsions of biologically active substances, used for production of functional meat-based curative-preventive products.

## II. MATERIALS AND METHODS

Systems water/oil (w/o) composed from plant (sunflower), wood (tall), animal (tallow) oils and water with inclusion of surface-active stabilizers (SUS) having "E" indices (mixes of carboxymethylcellulose, sodium alginate, carrageenan and guar gum) were used, added with 0.1 … 1.0% of vitamins and amino acids or without them.

A microgrinder PT2, ensuring mixing with rotation speed of agitator 2000 and 5000 rpm, the ultrasound (US) bath with power input US of 400 W/l during 1 … 2 hours and a mechanical disintegrator with coaxial rotating discs (rotation speed 160 rps ) with slots ensuring homogenization of solid and liquid media and mechanical and chemical destruction of large BAS were used for dispersion of systems.

Particle size was evaluated using an optical microscope "Jenaval" Carl Zeiss (Germany) – the objective X40 with the use of video-computer image analysis "Scion image, USA", allowing observation of objects with minimum particles size > 100 nm (0.1 μm), and the optical method of light dissipation according to Relay (Ivankin, Yushina, Gorbunova, Evdokimov, 2010). For this purpose, a 20% w/o microemulsion obtained according to technology, was diluted with water, with

preparation using the intensive mixing of 4 dilutions: an aliquot 10 μl + 20 ml of water (1: 2000), + 50μl of water (1: 5000), + 100 ml of water (1 : 10000), and then the extinction of the prepared diluted emulsions at 546 nm was measured on a photometer ECOM (Eppendorf, Germany) in a dish with the optical layer thickness 1cm. The calculation was conducted according to Relay formula [2]. The numerical compositions of amino acids and vitamins in the mixtures were estimated according to data of HPLC (Lisitsyn, Ivankin, Nekludov, 2002).

## III. RESULTS AND DISCUSSION

Tables 1-2 show distribution of particles by their size for microemulsions of different composition, estimated by the data of light microscopy.

Table 1

Influence of ultrasound treatment time of water-oil system 1:1 with 25 kHz frequency and temperature $40^0$C on size of microemulsion drops

| Time, min. | Average size of the main mass of particles, μm | | |
|---|---|---|---|
| | Sunflower oil | Tall oil | Pork fat |
| 0 | 24.3 ± 1.2 | 28.2 ± 1.6 | 25.6 ± 1.2 |
| 1 | 15.6 ± 0.7 | 14.9 ± 0.8 | 22.1 ± 1.5 |
| 3 | 4.2 ± 0.3 | 9.3 ± 0.4 | 18.5 ± 0.9 |
| 5 | 2.2 ± 0.2 | 6.1 ± 0.4 | 16.8 ± 0.8 |
| 7 | 2.0 ± 0.2 | 2.0 ± 0.2 | 9.7 ± 0.3 |
| 10 | 2.1 ± 0.2 | 2.1 ± 0.2 | 4.2 ± 0.2 |
| 15 | 2.0 ± 0.3 | 2.0 ± 0.3 | 2.0 ± 0.2 |
| 20 | 2.0 ± 0.2 | 1.9 ± 0.3 | 2.1 ± 0.2 |

Stabilized emulsions have particle sizes distributions from 0.1 to 200 μm depending on the efficiency of the dispersion device used.

Stabilization of the system with the used synergetic combination of SAS allowed obtaining of rheologically stable systems, preserving more than 95% of initial distribution of particles sizes during 3 … 6 months.

Table 2

Polydispersivity of visible part of 20% micronanoemulsion in water mixed with stabilizers (1% of sodium alginate, 1% of carboxymethylcellulose and 1% of guar gum)

| Name | Average diameter, μm (% from the sum of particles) | | | | |
|---|---|---|---|---|---|
| | 0.1…1.0 | 1.0…2.0 | 2.0…10.0 | 10.0…40.0 | 40…200 |
| Sunflower oil, mixer 5000 rpm, 1 hour | (22 %) | (22 %) | (40 %) | (13 %) | (3 %) |
| Sunflower oil, ultrasound, 200 Wt/l, 2 hours | (37 %) | (30 %) | (20 %) | (12 %) | (1 %) |
| Pork fat, disintegrator, 1 hour | (41 %) | (35 %) | (20 %) | ( 3 %) | (1 %) |
| Beef fat, mixer, 2000 rpm, 2 hours | (22 %) | (22 %) | (40 %) | (13 %) | (3 %) |
| Beef fat, ultrasound | (45 %) | (26 %) | (21 %) | (7 %) | (1 %) |

| | | | | | |
|---|---|---|---|---|---|
| 200 Wt/l, 2 hours | | | | | |

According to light microscopy the major mass of particles in the obtained emulsions has the size from 0.1 to 10 μm (Table 1). However, the total share of particles of less size 0.1 … 2 μm is also rather significant.

Formation of fat emulsion particles of vitamins and amino acids in strongly diluted solutions, containing natural polysaccharides, leads to production of systems with the size of their elements significantly less of the level, which light microscopy can test. The assessment of nanoparticles sizes in the obtained nanoemulsions shows, that their average size according to the data of light dissipation (Ivankin, Yushina, Gorbunova, 2010) is within 50 - 120 nm.

Introduction into the system of 0.1 % water-soluble vitamin C and 1 % fat-soluble vitamin $B_2$ allowed obtain immobilized forms of these vitamins. According to HPLC, as used for quantitative determination of vitamins [3], there are actually 100% of vitamin $B_2$ and more than 90% of vitamin C in lipid phase, which is associated with high efficiency of solubilization of BAS with the selected system of stabilization.

A similar picture was observed for more complex systems. For example, the equimolar mixture of amino acids Cys, Lys, His, Arg, Ser, Asp, Gln. Glu, Thr, Ala, Tyr, Val, Met, Phe, Ile and Leu, containing 50% mass of the added mixture of vitamins A, $B_1$, B2, PP and C were subjected to US immobilization in the presence of 0.5% of equimolar mixture of carragheenan, sodium alginate and gum in a 20% lipid mixture with water. As a result, micronanoemulsions were obtained in which immobilized forms of biologically active substances possessed stability to the effects of technological temperatures.

Table 3 shows some constants of decomposition velocities of major vitamins.

Stability of vitamins in micronanoemulsions

| Name | Losses of immobilized vitamins after sterilization, % of the initial content | Destruction velocity , $V \cdot 10^4$, (mg /ml · min) |
|---|---|---|
| Vitamin A | 1.8 | 8.4 ± 1.6 |
| Vitamin $B_1$ | 0.05 | 0.09 ± 0.02 |
| Vitamin $B_2$ | 7.5 | 11.3 ± 1.1 |
| Vitamin C | 19.3 | 44.0 ± 6.5 |
| Vitamin PP | 1.7 | 3.8 ± 0.5 |
| Vitamin E | 11.1 | 17.3 ± 2.6 |

Preliminary investigations have shown that with the levels of vitamins $B_1$, $B_2$ and $B_3$ in 0.5 – 1.5 mg / 100 g of raw materials in ground meat, in the manufacture of ham products after thermal treatment during 1 hour at $70^0$C, the losses were (%): - 10, - 18 and -15%, respectively for $B_1$, $B_2$, and $B_3$. Further losses of vitamins during 1 month storage of products at+$2^0$C constituted (%): -2, -2 and – 1%, respectively.

Preliminary vitamins supplementation of ground meat for ham with microananoemulsion of the mixture of immobilized vitamins has shown, that, for example, for $B_1$ and $B_2$ groups of vitamins with their level of 2 – 25 mg / 100 g of product the losses were % (respectively) – 2 and – 4 %, and subsequent losses of these vitamins during storage of products (3 months) at +$4^0$C were (%) -0 and – 1%, while the losses of non-immobilized vitamins during sterilization constituted 5 – 40%, additionally increasing in storage by 10-15%.

## IV. CONCLUSION

Thus, the conducted investigations confirmed a relative stability of micronanoemulsions of the mixture of major vitamins to temperature effects, both in model experiments and in the tests on meat products.

## References

A,D, Nekludov, A.N. Ivankin (2003). Biologically active compounds from natural objects. Properties and structural-functional interrelationships. – M. Moscow State Forest University, 480 pp.

A.N. Ivankin, Yu.K. Yushina, N.A. Gorbunova, Yu.M. Evdokimov, G.L. Oliferenko (2010). Nanomicrotechnologies of inclusion of active ingredients into food compositions, Meat industry, №1, pp. 23-25

3. A.B. Lisitsyn, A.N. Ivankin, A.D. Nekludov (2002). Methods of practical biotechnology. Analysis of components and microscopic amounts of admixtures in meat and other food products. – M. VNIIMP, 408 pp.