Functional curd product of increased biological value

V V Kryuchkova¹, I F Gorlov¹², M I Slozhenkina¹², N V Lomonova² and S N Belik³

¹ Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production, 6, Rokossovskogo street, 400131, Volgograd, Russian Federation
² Volgograd State Technical University, 28 Lenin avenue, 400131, Volgograd, Russian Federation
³ Rostov State Medical University, 29, Nakhichevansky Lane, 344022, Rostov-on-Don, Russian Federation

E-mail: kverav@yandex.ru

Abstract. The authors have theoretically proved and practically confirmed the feasibility of sesame seeds and whey proteins being used in manufacturing a curd product. The composition and properties of sesame seeds and whey proteins, as well as their doses, method, and a processing step to add them were studied; nutritional and biological values of the product were determined. The sensory points and physicochemical indices of the developed curd product were found to be significantly higher than ones of the control sample; the essential amino acids exceeded the reference protein by 26.5% in terms of six essential amino acids; the biological value made 65.3%; and the utility coefficient was 0.77, which proved the balance of the amino acid composition of the protein in the product developed and the body’s ability to use it more rationally.

1. Relevance
In the current difficult epidemiological and economic situation, when personal income decreases, the demand for natural fermented milk products remains and even is predicted to grow. Meanwhile, the demand for fermented milk products, containing non-dairy fat, decreased considerably (by 10-20%) and indicated a growing trend for healthy eating. In this regard, functional nutrition is of particular importance. The main purpose of functional products (FP) is to preserve, strengthen, or restore the health of consumers. In Russia, more than 35% of FP are dairy (including curd) products [1, 2]. The curd production is based on the coagulation of macromolecular aggregates of milk casein in order to form a clot, so curd is a concentrated protein product that has a number of advantages, including antioxidant, anticarcinogenic, anti-inflammatory, hypoglycemic, probiotic, anti-atherosclerotic, and geroprotective effects [3, 4]. In the production of functional foods, special attention is paid to the enriching components. The spices—paprika, coriander seeds, curcumin, and ginger—used in the FP production help reduce oxidative stress and inflammatory reactions, which was theoretically substantiated and practically proven [5, 6]. Special attention was paid to plant ingredients (berries, black and red currants, dogwood, blueberries, blueberries, pomegranates, cranberries, citrus fruits, etc.) that allow supplementing the main product with vitamins, microelements, and polyphenolic compounds. The ability of these components to reduce the oxidative stress markers (superoxide dismutase (SOD), oxidized LDL (oxLDL), glutathione peroxidase (GPx), and catalase) and the concentration of pro-inflammatory cytokines (TNF-
α, IL-6, and IL-8) were scientifically proven [7, 8, 9]. Thus, the development of a curd FP enriched with ingredients of plant and animal origin is relevant and promising from the scientific and practical points of view.

2. Materials and methods

Experimental studies were conducted in laboratories at Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production (Volgograd), Volgograd State Technical University, and Research Testing Laboratory “Nika and K” (Rostov-on-Don). The objects of the research were natural cow’s milk of not lower than 1st grade according to GOST 31449-2013, acidity of not more than 19°T, and density of not less than 1.028 g/cm³; fat-free curd with acidity of from 170-240°T, f.i.d.m. of 1.8%, and dry solids weight ratio of 80% according to GOST 31453-2013; culture “Curd” VIVO (manufactured by LLC “VIVO”), specs 9223-001-18137828-2015; sesame seeds; whey proteins; and enriched curd product. When organizing and conducting the research, there were used generally accepted standard and modified research methods, i.e. sensory, physicochemical, and biochemical procedures. Basic research methods were as follows: determination of fat content according to GOST R ISO 2446-2011; protein determination by the Kjeldahl method according to GOST 34454-2018; determination of dry substance in curd by the accelerated method according to GOST 3626-73; sensory evaluation was performed in accordance with GOST R ISO 22935-2-2011; microbiological points were determined according to GOST 32901-2014; and the amino acid compositions of sesame seeds, whey proteins, and curd product were found by capillary electrophoresis using the Kapel-105M system. The data obtained were statistically processed using the Statistica 6.0 software package. The experiments were repeated three times.

3. Research results

Sesame seeds and whey protein were selected as functional ingredients for developing a curd product. Sesame is an annual herb; its seeds are of high nutritional value. Sesame seeds have a high oil content of 40-60%, including equal portions of oleic (from 35 to 54%) and linoleic (from 39 to 59%) acids. The sesame seeds also contain 10% of palmitic acid and 5% of stearic acid [10] and up to 20% of protein-limited lysine that is rich in tryptophan and methionine [11]. The high oxidative stability of sesame seeds is due to various biologically active components, including lignans, phytosterols, and tocopherols. The main lignans of sesame seeds are water-soluble lignan glycosides (sesaminoltriglucoside and sesamoldiglucoside) and lipid-soluble lignans (sesamin and sesamolin)—groups of phenolic compounds of plant origin [12, 13]. Phenolic compounds are known to exhibit antioxidant activity—they bind heavy metal ions and serve as free radical scavengers [14]. Sesame lignans have a significantly higher percentage (p<0.05) of inhibition of linoleic acid oxidation (53.6%) compared to α-tocopherol (45.0%), which confirms the high potential of the antioxidant activity of this product [15]. The sesame seeds corresponded to standard sesame seeds in terms of color, taste, and smell, i.e. without musty or other foreign smells and aftertastes. Nutritional value, amino and fatty acid, and vitamin and mineral compositions of sesame seeds are presented in tables 1, 2 and 3.

| Table 1. The nutritional value of sesame seeds. |
|-----------------------------------------------|
| Parameter                                      | Value  |
| Weight fraction of fat, g, incl.              | 49.7±0.19 |
| - unsaturated fatty acids                      | 40.6±0.21  |
| - saturated fatty acids                        | 7.0±0.09   |
| Weight fraction of protein, g, incl.           | 17.7±0.1   |
| - essential amino acids                        | 8.0±0.12   |
| - nonessential amino acids                     | 9.6±0.08   |
| Weight fraction of carbohydrates, g, incl.     | 23.6±0.18  |
| - dietary fiber, g                             | 5.6±0.10   |
| - pectin                                       | 0.4±0.01   |
| Weight fraction of moisture, g                 | 9.0±0.11   |
| Total                                         | 100.0±0.17 |
The study found that fats (49.7%) and carbohydrates (23.6%), including dietary fiber of 5.6%, predominated in sesame seeds, which is very important for manufacturing functional products.

Table 2. The contents of amino and fatty acids in sesame seeds.

| Amino acid            | Content, g per 100 g of product | Amino acid            | Content, g per 100 g of product |
|-----------------------|---------------------------------|-----------------------|---------------------------------|
| Essential amino acids |                                 | Nonessential amino acids |                                |
| Valine                | 1.10±0.02                       | Alanin                | 1.10±0.04                       |
| Histidine             | 0.6±0.02                        | Arginine              | 3.10±0.21                       |
| Isoleucine            | 0.90±0.03                       | Aspartic acid         | 1.90±0.11                       |
| Leucine               | 1.60±0.1                        | Glycine               | 1.40±0.01                       |
| Methionine            | 0.70±0.05                       | Glutamic acid         | 4.70±0.01                       |
| Threonine             | 0.87±0.121                      | Proline               | 0.97±0.07                       |
| Tryptophan            | 0.47±0.1                        | Serine                | 1.10±0.05                       |
| Phenylalanine         | 1.10±0.03                       | Tyrosine              | 0.87±0.05                       |
| ∑ essential amino acids | 8.01±0.15                      | Cystine               | 0.44±0.07                       |
|                       |                                | ∑ non-essential amino acids | 15.58±0.12                      |
| Total, essential + nonessential amino acids |                  |                       | 23.59±0.14                      |
| Saturated fatty acids |                                 | Unsaturated fatty acids |                                |
| Myristic C 14:0       | 0.13                            | Palmitoleic C 16:1 (omega-7) | 0.15                           |
| Palmitic C 16:0       | 4.44                            | Oleic C 18:1 (omega-9) | 18.52                           |
| Stearic C 18:0        | 2.09                            | Linoleic C 18:2 (omega-6) | 21.37                           |
| Total, saturated      | 6.66                            | Linolenic C 18:3 (omega-3) | 0.38                           |
|                       |                                 | Gadoleic C 20:1 (omega-11) | 0.07                           |
|                       |                                 | Total, unsaturated    | 40.49                           |
| Total, saturated + unsaturated |              |                       | 47.15                           |

Sesame seeds were found to contain large quantities of both essential and nonessential amino acids (37.0% and 27.4% of their daily rate in 100 g of seeds, respectively), while the contents of all amino acids in the seeds (100 g) exceeded 10% of the daily intake. Sesame were especially rich in essential amino acids, such as tryptophan (47%), valine (46%), and isoleucine (45%); arginine (50.8%) and glycine (40%) dominated among the nonessential amino acids.

Unsaturated fatty acids (99%) dominated in sesame seeds, especially polyunsaturated fatty acids—linoleic acid (omega-6); its content in 100 g made 213.7% of the daily intake. The ratio between the contents of polyunsaturated fatty acids—omega-6 and omega-3—was 5.7:1, which was optimal for the human body. Sesame also contained a lot of monounsaturated oleic acid (omega-9) (18.52 g) that protects blood vessels from the cholesterol plaques and is a good preventive agent against atherosclerosis.

Table 3. Vitamin and mineral compositions of sesame seeds and their percentages of the daily value.

| Name                  | Weight, mg per 100 g of product | % of daily value | Name                  | Weight, mg per 100 g of product | % of daily value |
|-----------------------|---------------------------------|-----------------|-----------------------|---------------------------------|-----------------|
| Vitamins              |                                 |                 | Minerals              |                                 |                 |
| Vitamin B₁ (thiamine), mg | 0.79                           | 46.8            | Potassium, mg         | 482.5                           | 19.2            |
| Vitamin B₂ (riboflavin), mg | 0.25                           | 12.5            | Calcium, mg           | 1128.5                          | 113.6           |
Analysis of the vitamin and mineral compositions of sesame seeds showed considerable amounts of vitamins B₁ (46.8% of the daily intake), B₆ (39.5%), folic acid (24.3%), PP (22.6%), and B₁₂ (12.5%).

The basis of the mineral composition of sesame seeds is made of silicon (663.3% of the daily intake), copper (410.0%), vanadium (136.8%), nickel (126.7%), manganese (123.0%), calcium (113.6%), iron (96.7%), magnesium (87.8%), phosphorus (67.5%), zinc (64.6%), boron (52.9%), zirconium (50.0%), selenium (37.5%), molybdenum (21.4%), cobalt (20.0%), potassium (19.2%), iodine (8.9%), etc.

Based on the foregoing, sesame seeds used in the production of a functional curd product for all groups of consumers are promising and commercially successful.

We also proposed whey protein as a functional ingredient to enrich a new curd product. In the curd production, whey proteins (0.6%) remain in whey after the removal of casein; they make up about 20% of all milk proteins and include 52% of β-lactoglobulin (β-LG), 23% of α-lactalbumin (α-LA), 10-15% of bovine serum albumin (BSA), 16% of immunoglobulins (IG), 8% of serum albumin, and 1% of lactoferrin and other minor proteins.

The β-LG is a retinol-binding protein and determines the quantitative content of vitamin A in milk and whey; moreover, it is a source of essential amino acids, including sulfur-containing amino acids. The β-LB is noted for the highest immunoreactivity among whey proteins, which is due to its resistance to proteolysis by pepsin in the acidic environment of the stomach [16, 17]. The α-LA contains a wide range of essential amino acids; therefore, its purified form is widely used in the manufacture of infant milk formula to make their protein composition and biological properties similar to human milk. The α-LA possesses antitumor, antimicrobial, antioxidative, and antiviral activities, as well as immunomodulatory and antistress effects [18]. Bovine serum albumin (BSA) is able to suppress the proliferation of cancer cells by changing the activity of autocrine regulatory factors; it also protects anchored lipids from oxidative modification [19]. The IGs obtained from whey fractions in conjunction with serum minerals have a pronounced immunostimulating effect, anticarcinogenic and antioxidant activities. They are able to arrest metabolic stress, increase emotional stress resistance, improve muscle functionality, and have a positive effect on health in general [20, 21]. Whey protein quality indicators are presented in table 4.

| Indicator                     | Characteristic                                      |
|-------------------------------|-----------------------------------------------------|
| Appearance and texture        | Soft, spreadable, slightly crumbly texture with milk protein particles |

Table 4. Whey protein quality indicators.
The table shows that the whey proteins had rather high consumer properties. Whey proteins contained more essential amino acids than casein; therefore, from the point of view of the nutritional physiology, they should be considered the most complete (figure 1). Whey proteins contained more sulfur than casein, which indicated sulfur-containing amino acids-cystine and cysteine.

Figure 1 shows that the content of essential amino acids in whey proteins exceeded the number of essential amino acids in the reference—chicken protein—by 25%; the number of non-essential amino acids exceeded the number of nonessential amino acids in the reference by 5%. The total amino acids value in whey proteins was by 16.5% more than in the reference and by 3.1% more than in casein.

The amino acid tryptophan is very important; its content in whey proteins was 2.5 times more than in the reference and by 39% more than in casein.

Thus, the conducted studies of the qualitative characteristics of whey proteins allowed us to conclude that they have good consumer properties and high physicochemical and microbiological indices; the amount of essential amino acids in whey proteins exceeds the number of essential amino acids in terms of all essential amino acids, except for valine and phenylalanine. Hence, it follows that the whey proteins added into the formulation of curd products cause an improvement in the consumer and technological properties of fortified products.

The whey proteins were found to be rapidly digestible. The difference in the assimilation rate and the effect on the metabolism in the body between proteins casein and whey milk proteins can be explained by peculiarities of their amino acid compositions and different influence of various amino acids on the secretion of insulin and glucagon. It was also found that whey proteins have anticarcinogenic and immunomodulatory properties, antimicrobial activity, as well as anti-inflammatory and toxin-binding effects; they also positively effect on the growth of bifidobacteria. These properties

| Taste and smell | Pure fermented milk with a pronounced taste of pasteurized milk |
|----------------|-------------------------------------------------------------|
| Colour         | Cream, uniform throughout the mass                           |

| Physical and chemical characteristics                                    |
|-------------------------------------------------------------------------|
| Weight fraction of moisture, %                                          | 73.0±2.3                                                   |
| Titratable acidity, °T                                                  | 164±1.4                                                    |
| Temperature, °C                                                         | 4±2                                                         |
| Phosphatase                                                             | Not found                                                   |

### Essential amino acids in the reference (chicken) protein, whey protein, and casein

![Bar graph showing the comparison of essential amino acids in the reference, whey protein, and casein]

**Figure 1.** Essential amino acids in the reference (chicken protein), whey proteins, and casein.
of whey proteins can be explained by better utilization and digestibility of these proteins; α-lactalbumin destroys malignant tumors in the head, neck, and digestive tract, thereby supporting the growth of normal cells.

Therefore, whey proteins as a functional additive in curd products make it possible to obtain a product with good consumer properties, which is relevant and expedient.

The whey proteins were produced from whey obtained in the production of fat-free curd, containing 0.6% of whey proteins. We provided for the production of whey proteins by sparging with live steam at a temperature of 90-95 °C for 1.5-2 hours, separating the whey, self-pressing, and cooling to a temperature of 14 °C.

To calculate the amount of whey proteins, it was necessary to calculate the amount of milk used to obtain 1 ton of non-fat curd, taking into account the effective amount of dry matter in skim curd, whey, and skim milk and considering losses due to the formula:

\[
M_{sm} = \frac{DM_c - DM_w}{DM_{sm}(1 - 0.01L) - DM_w}
\]

where \(M_{sm}\) is the amount of skim milk, necessary to obtain 1 ton of skim curd;
\(DM_c\) is the dry matter content in skim curd, %;
\(DM_w\) is the dry matter content in whey, %;
\(DM_{sm}\) is the dry matter content in skim milk, %; and
\(L\) is the loss coefficient in the production of skim curd (3.32%).

According to the calculation, to produce 1 ton of skim curd we need 7793 kg of milk; 80% of the milk is whey used for the production of whey proteins. Further calculation showed that 32.6 kg of whey proteins are obtained per 1 ton of skim curd, taking into account the losses; therefore, 3.0% of whey proteins is used in the production of an enriched curd product. So, when developing an enriched curd product, we use this exact amount of whey proteins.

To develop a technology for the fermented milk product, it was necessary to establish a method and determine a processing step for whey proteins and sesame seeds to be added.

The analysis of the table showed that Mode II of processing sesame seeds with hot milk at a temperature of 75±2°C for 25-30 minutes ensured high microbiological indices and no extraneous microflora. Sesame seeds acquired a pleasant pronounced taste of pasteurized milk, harmoniously complemented with the fermented milk taste of the curd base.

Table 5. Microbiological points of sesame seeds, depending on the temperature-time regime of the heat treatment.

| Parameter                  | Product weight (g) that does not allow | Value / temperature-time regime |
|----------------------------|----------------------------------------|---------------------------------|
|                            |                                        | \(t=65±2°C\) | \(t=75±2°C\) | \(t=85±2°C\) |
|                            |                                        | \(\tau=25-30\) min | \(\tau=25-30\) min | \(\tau=25-30\) min |
| Pathogenic microorganisms, incl. salmonell {L}isteriamonocytogenes {E}nterobactersakazaki {Y}ersinia bacteria | 25 | not found | not found | not found | not found |
| Mold, CFU/g                 | 50                                     | 15                             | not found | not found | not found |
| Yeast, CFU/g                | 50                                     | 12                             | not found | not found | not found |

Manufacturing the enriched curd product involves adding prepared sesame seeds at the mixing stage.
Based on the results obtained, there was developed a technology of an enriched curd product that provided for the preparation of a setting to be mixed in a rotary-pulsed apparatus. The setting included skim curd and functional ingredients - whey proteins and sesame seeds - to obtain a product of the required texture, with improved taste characteristics, high biological value, and increased stability during storage according to the HACCP system. The diagram process flow for the curd product is shown in figure 2.

**Figure 2.** Diagram process flow for an enriched curd product according to the HACCP system (where S is the sensory indicators, i.e. appearance, texture, taste, smell, and colour; PhCh is the physical and chemical indicators, namely, weight fractions of fat, protein, and moisture, density, particulate contamination, temperature, holding time, and weight of the product; M is the microbiological points, i.e. total contamination, the numbers of somatic cells, lactic acid bacteria, pathogenic and opportunistic microorganisms, yeast, and mold; and H is the hazards that involve toxic elements, nitrates, nitrites, radionuclides, and mycotoxins).
The sensory, physicochemical, and microbiological indicators of the enriched curd product are presented in Table 6.

**Table 6. Sensory and physicochemical characteristics of curd products.**

| Indicator | Control sample | Enriched curd product |
|-----------|----------------|-----------------------|
| **Sensory characteristics** | | |
| Appearance and texture | Soft, homogeneous, thick texture | Soft, spreadable, slightly crumbly texture with single sesame seeds |
| Taste and smell | Pure fermented milk taste and smell, without foreign aftertastes | Pure, fermented milk with a pronounced aftertaste of pasteurized milk and sesame seeds harmoniously complimented |
| Colour | Milky-white, uniform throughout the mass | Light cream, heterogeneous, with occasional sesame seeds and whey proteins, evenly distributed throughout the mass |
| **Physical and chemical characteristics** | | |
| Weight fraction of fat, %, not less than | 18.0±0.3 | 18.5±0.9 |
| Weight fraction of protein, % | 9.0±0.2 | 10.0±0.3 |
| Weight fraction of moisture, %, no more than | 75.0±1.5 | 72.0±1.2 |
| Weight fraction of sesame seeds, % | - | - |
| Titratable acidity, °T | 162±0.2 | 160±0.1 |
| Phosphatase | Not found | |
| Energy value, kcal | 198.0±2.1 | 206.5±1.3 |
| **Microbiological points** | | |
| The number of lactic acid bacteria at the end of the shelf life, CFU/g | 1*10⁶ | 2*10⁹ |
| Product weight (g) that does not allow | Coliform bacteria | not found |
| | Staphylococci S. aureus | not found |
| | Pathogenic (incl. salmonella) | not found |

The sensory, physicochemical, and microbiological indicators of the enriched curd product are presented in Table 7.

**Table 7. Sensory and physicochemical characteristics of curd products.**

| Indicator | Control sample | Enriched curd product |
|-----------|----------------|-----------------------|
| **Sensory characteristics** | | |
| Appearance and texture | Soft, homogeneous, thick texture | Soft, spreadable, slightly crumbly texture with single sesame seeds |
| Taste and smell | Pure fermented milk taste and smell, without foreign aftertastes | Pure, fermented milk with a pronounced aftertaste of pasteurized milk and sesame seeds harmoniously complimented |
| Colour | Milky-white, uniform throughout the mass | Light cream, heterogeneous, with occasional sesame seeds and whey proteins, evenly distributed throughout the mass |
| **Physical and chemical characteristics** | | |
| Weight fraction of fat, %, not less than | 18.0±0.3 | 18.5±0.9 |
| Weight fraction of protein, % | 9.0±0.2 | 10.0±0.3 |
The study results showed that the fortified curd product had higher sensory characteristics than the control sample. Due to whey proteins and sesame seeds added into the product, the fat content increased by 0.5% and content of proteins by 1.0%, including 0.8% of whey protein, which indicated an increased nutritional value of the fortified curd product.

Table 8. Biological value of enriched curd product (ECP).

| Name    | FAO reference, (g/100 g of protein) | OTII (g/100 g of protein), (p≤0.05) | Amino acid score, % | ΔRAS | KRAS | BV | K_{i} | U |
|---------|------------------------------------|-------------------------------------|---------------------|------|------|----|------|----|
| Valine  | 5.2                                | 5.7                                 | 109.6               | 13.8 |      |    | 0.87 |    |
| Lysine  | 5.5                                | 8.8                                 | 160.0               | 64.2 |      |    | 0.60 |    |
| Methionine | 2.8                             | 2.7                                 | 96.4                | 0.6  |      |    | 0.99 |    |
| Phenylalanine | 4.8                            | 4.6                                 | 95.8                | 0    |      |    | 1.0  |    |
| Threonine | 3.7                               | 4.8                                 | 129.7               | 33.9 |      |    | 0.74 |    |
| Isoleucine | 4.6                              | 5.4                                 | 117.4               | 21.6 |      |    | 0.82 |    |
| Tryptophan | 1.0                             | 1.95                                | 195.0               | 99.2 |      |    | 0.49 |    |
| Leucine  | 6.9                                | 9.7                                 | 140.6               | 44.7 |      |    | 0.68 |    |
| Total   | 34.5                               | 43.65                                | 277.9               |      |      |    | 6.20 |    |

Microbiological points characterized curd products as living products due to lactic acid bacteria in an amount of 2*10^6 CFU/g and as safe products due to the absence of pathogenic and opportunistic microorganisms, such as Coliform bacteria, *staphylococcus S. aureus*, or *Salmonella bacteria*, which corresponded to the TR CU requirements 033/2013 for this group of fermented milk products. Table 7 shows the results of calculating the biological value of the enriched curd product.

Methionine was found to be the limiting amino acid in the enriched curd product; its amino acid score was 95.8%. The enriched curd product contained more essential amino acids than the reference protein by 26.5% and higher values of six essential amino acids, such as valine (by 9.6%), lysine (by 60.0%), threonine (by 29.7%), isoleucine (by 17.4%), and leucine (by 40.6%); tryptophan almost twice exceeded the values of the reference.

The calculation of the biological value (BV) of the enriched curd product made 65.3%, which characterized it as a product with a high biological value.

The utilitarian coefficient of the amino acid composition (U) of 0.77 proved the balanced amino acid composition of the enriched curd product, since the higher the value of the utilitarian coefficient is, the better balanced the amino acids in the protein are and the more rationally they can be used by the body.
4. Conclusions and recommendations

The authors studied the compositions and properties of sesame seeds and milk whey protein and established their high nutritional and biological values. Their high functional activity was shown. There was found a method to produce whey proteins from whey obtained in the curd production, functional ingredients pretreated, doses of whey proteins and sesame seeds added, and processing step to add them into the prepared curd base, i.e. the mixing stage. The technology of enriched curd product was developed according to the HACCP system. The sensory and physicochemical indices of the enriched curd product were found to be significantly higher than in the control sample—an additive-free curd product. Enrichment of a curd product with whey proteins and sesame seeds increased its nutritional and biological value. The number of essential amino acids exceeded the value of the reference protein by 26.5% for six amino acids, the biological value was 65.3%, and the utility coefficient was 0.77, which proved the balance of the amino acid composition of the protein of the enriched curd product, so they can be used more rationally by the body.

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