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ISOMOLAR SERIES METHODOLOGY IN SENSORY ANALYSIS OF FISH CULINARY PRODUCTS FOR HEALTHY-CAFE

The object of research is the technology of fish products for HEALTHY-CAFE with regulated histamine content for the development of health food diets for the population. One of the most problematic places in the technology of food products from raw materials of aquatic origin is microbiological spoilage and, as a result, the formation and accumulation of HisA, which in a certain amount causes a toxic effect.

In the course of the study, the methods of isomolar series, sensory analysis, and the study of quality indicators were used. The methodology chosen in the work allows to determine the optimal ratio of hydrocolloids in the system for maximum complexation with histamine in the technology of fish culinary products in gelatin fillings with a harmonious sensory profile and adjustable histamine content.

The obtained results of the conducted research allow to state that the proposed method of determining the optimal ratio of sodium alginate and low-esterified pectin substances contributes to the development of a gelatinous filling for fish culinary products in order to ensure the harmonious flavor of ready-made fish dishes and contributes to the expansion of the range of fish products for HEALTHY-CAFE with functional and preventive properties. This is due to the fact that taking into account the modern trends in nutrition regarding the safety, functionality, palatability, and attractiveness of fish food products made it possible, based on the method of isomolar series and sensory analysis, to scientifically substantiate the optimal ratio of plant biopolymers, to form requirements for the texture of gelatin filling in fish technology culinary products. On the basis of previous experimental studies, it was shown that the accumulation of histamine occurs more actively in sea fish, which made it possible to substantiate the choice of raw materials for the production of fish culinary products. Taking into account the main global trends in the development of aquaculture, it is proposed to use crucian carp as a raw material, as the main object of aquaculture in Ukraine. The low activity of the peptide hydrolase complex of the muscle tissue of the carp, compared to sea fish, contributes to the formation of a harmonious aromatic profile of fish culinary products, which corresponds to consumer expectations, and the use of natural hydrocolloids of vegetable origin to form a gelatinous structure provides functional properties to the food product and allows controlling the histamine content.

Keywords: *isomolar series, sensory analysis, fish products, HEALTHY-CAFE, nutritional value, histamine, complex formation, hydrocolloids.*

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1. Introduction

The peculiarities of raw materials and the technology of food production from hydrobionts in many cases lead to the formation of an excess amount of histamine that exceeds permissible standards. According to regulatory documents, the maximum permissible level of histamine for Ukrainian producers of fish products for raw materials and finished products is 100 mg/kg [1]. In the USA and Canada, up to 50 mg/kg is allowed, in Australia – up to 100 mg/kg, in Sweden – up to 100 mg/kg in fresh fish and no more than 200 mg/kg in salted fish. Regulatory documents of the USA allow the content of histamine in food products to be 50 mg/100 g, in Canada, Denmark,

India, Sweden the maximum permissible levels are 20 mg. A pseudoallergic reaction can be caused by products with a histamine content of 5–10 mg/kg [2]. Histamine is a biogenic amine, a mediator of the nervous system, a hormone [3]. A breakdown product of one of the amino acids, histidine. Under the influence of histidine decarboxylase and with the participation of vitamin B6, the carboxyl tail is separated from histidine, thus the amino acid turns into an amine [4]. Food products that are characterized by a high content of histamine are, as a rule, of animal origin. These include fermented meat and fish products with a long shelf life, hard and processed cheeses, all legumes, nuts, citrus fruits, etc. The longer the product is stored, the more histamine it contains [5–9]. Biogenic amines (BAs) have various toxicological

effects on human health and are associated with numerous foodborne disease outbreaks. BAs are also known to cause cancer due to their ability to react with nitrite salts, resulting in the formation of carcinogenic organic compounds (nitrosamines). Ingestion of large amounts of BA in food causes toxic effects and health disorders, including psychoactive, vasoactive, and hypertensive effects, as well as respiratory, gastrointestinal, cardiovascular, and neurological disorders. BA toxicity is closely related to histamine [10]. The tolerance limit of histamine for an adult is 5–6 mg/kg [11, 12]. The toxic dose is defined as >100–1000 mg/kg of the product, and the highly toxic dose is more than 1 g/kg. The technology of many food products of animal origin is characterized by a tendency to use biopolymers of plant origin as structure-builders and to provide functional properties of finished products, which corresponds to the main modern trends in nutrition.

Today, a healthy diet is understood as promoting health and preventing diseases through the consumption of food products. Such nutrition provides an adequate, without excess, supply of nutrients and substances that promote health, and allows to avoid the consumption of substances that harm health [13]. That is, modern food technologies must ensure the safety of food products, which is understood as «all those dangers, chronic or acute, that can harm the health of the consumer» [14]. Food safety problems can arise as a result of contamination of food products with biological hazards, pathogens, and chemical substances [13, 15, 16].

Histamine accumulates during storage and technological processing of hydrobionts [17, 18]. The formation of biogenic amines in fish and fish products is associated with the action of microorganisms that have decarboxylase activity. These include *Morganella morganii*, *Morganella psychrotolerans*, *Hafnia alvei*, *Photobacterium phosphoreum*, *Photobacterium psychrotolerans*, *Klebsiella pneumoniae*, *Clostridium spp.*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas cepaciae*, *Aeromonas spp.*, *Aeromonas hydrophila*, *Acinetobacter lawffi*, *Plesiomonas shigelloides*, *Proteus vulgaris*, *Proteus mirabilis*, *Serratia fonticola*, *Serratia liquefaciens*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Citrobacter freundii*, *Raoultella planticola*, *Staphylococcus xylosum*, *Staphylococcus epidermidis*, *Bacillus spp.*, *Vibrio alginolyticus*, *Vibrio spp.*, *Escherichia*, etc. [19–21].

Due to its toxicity, histamine is a unique biogenic amine, the content of which in fish products is limited by regulatory documents [1, 22]. Poisonings associated with high levels of histamine occur worldwide. The largest number of poisoning cases was described in the USA, Great Britain, Australia and Japan. There is an assumption that up to 40 % of food poisoning outbreaks registered in Europe and the USA may be associated with histamine intoxication [23]. Scientists have even introduced the concept of a «low-histamine diet», which is currently the most recommended strategy for preventing the symptoms of histamine intolerance [24]. Therefore, studies devoted to the development of methods of regulating the content of histamine in fish and fish products are relevant.

In recent years, consumers have become increasingly demanding about the quality, safety and functionality of food products. Consumption of foods containing large amounts of histamine and other biogenic amines can cause food poisoning with various symptoms related to individual sensitivity and detoxification activity. Histamine is the only biogenic amine whose content in fish and fish products

is limited by the European Commission because it can lead to death [25].

It is known that histamine in food causes food poisoning and allergic reactions. Let's usually get histamine from cooked food, but there is little research on the effect of cooking method on histamine levels [26]. Therefore, methods of regulating the content of histamine in fish products are gaining special relevance. One of the technological techniques is the introduction of natural biopolymers of plant origin into the recipe, which has complex-forming properties. The authors used this method in the technology of fish snacks, which allowed to reduce the content of free histamine in finished products [27].

Another example of the use of hydrocolloids is the production of fish culinary products in jelly fillings [28–30].

There are known works on the research of rheological properties of stabilization systems for canned fish products [31]. The authors investigated the structural and mechanical properties of stabilization systems; established the dependence of the coefficients of the limit load and molecular adhesion on the concentration of the mixture in the system, but the optimal ratio of hydrocolloids has not been determined.

The development of restructured fish products using vegetable polymers is known [32]. The influence of hydrocolloids-synergists, which perform the functions of stabilizers in the formulation of gelling fillings for canned fish for the formation of a strong and stable jelly during the storage of canned fish, was investigated [33].

However, the analysis of literary sources shows that there is practically no information on the complex formation of hydrocolloids with histamine in food products from water raw materials, which requires conducting systematic studies and evaluating the impact of technological processes on the complex formation of low-esterified pectin substances and alginates with histamine. Solving this issue will allow to create safe fish food products with high organoleptic qualities and functional properties of preventive orientation.

Therefore, the purpose of the study is to determine the complex-forming ability of the studied hydrocolloids with respect to HisA for their use in the technology of fish culinary products in gelatin filling to expand the HEALTHY-CAFE range. This will make it possible to create safe fish culinary products with additional functionality, which corresponds to modern nutritional trends regarding food products with a preventive effect.

To achieve the aim, the following objectives are set:

1. To study the comparative UV spectra of NPR, AlgNa solutions and a mixture of NPR+AlgNa hydrocolloids with histamine to determine the maxima.
2. To determine the stability constants of the formed NPR, AlgNa, NPR-AlgNa complexes with histamine.
3. To determine the composition of the composition of hydrocolloids, which corresponds to the maximum complex-forming ability.

2. Materials and Methods

The object of research is the technology of fish products for HEALTHY-CAFE with regulated histamine content for the development of health food diets for the population. The subject of research is the ratio of hydrocolloids (low-esterified pectin substances and sodium alginate) for the

production of gel filling, the complexing ability of hydrocolloids in relation to histamine, the stability constants of complexes.

A feature of the technology of fish culinary products is the preparation of jelly filling. For the formation of a gelling composition, a finely dispersed powder of low-esterified apple pectin with a degree of esterification of 35 % of the brand NH Nappage (thermo-reversible), Louis Francois was used; sodium alginate of the Danisco FD 127 brand. As a source of calcium ions, a 10 % solution of calcium chloride was used according to TU 21.2-25657043-069-2014. The process of jelly formation was studied at a temperature of 20 °C with the measurement of jelly strength using a modified Valenta device (manufactured by Poland) according to GOST 26185-84, which determines the mass of the sample required for the destruction of the jelly. To prevent sticking and partial dissolution of pectin substances, it is important to ensure dispersion of particles of dry pectin powder. The technology for the preparation of the filling involves the preparation of swollen hydrocolloids, mixing the swollen mixture of structure-formers with a previously prepared spicy broth, heating the resulting solution, adding a hot 10 % calcium chloride solution.

The composition of complex compounds was determined by the methods of isomolar series and molar ratios, which is based on determining the ratio of isomolar concentrations of the interaction of hydrocolloids with histamine, which corresponds to the maximum yield of the complex compounds formed. At the same time, the curve of dependence of the yield of the complex and, thus, the change in signal intensity on the composition of the solution is characterized by the extremum point. Such a point indicates the maximum possible concentration of the complex, and its position on the abscissa axis corresponds to the stoichiometric ratio of reagents, by establishing the dependence of the change in optical density on the concentration of one of the components at a constant concentration of the second component. Thus, it is possible to determine the rational composition of complex compounds for maximum complex formation with free histamine.

To study the complexing ability of hydrocolloids in relation to histamine, the method of isomolar series was used.

Model solutions of de-esterified apple pectin (NPR), sodium alginate (AlgNa) and a mixture of hydrocolloids (NPR+AlgNa) with histamine were tested. The complexes were obtained at a temperature of 18–20 °C by merging aqueous solutions, respectively.

The composition of complex compounds was determined by the methods of isomolar series and molar ratios [34]. The method of isomolar series is based on the determination of the ratio of isomolar concentrations of the interaction of hydrocolloids with histamine, which corresponds to the maximum yield of the complex compounds formed. At the same time, the curve of dependence of the yield of the complex and, thus, the change in signal intensity on the composition of the solution is characterized by the extremum point. Such a point indicates the maximum possible concentration of the complex, and its position on the abscissa axis corresponds to the stoichiometric ratio of reagents, by establishing the dependence of the change in optical density on the concentration of one of the components at a constant concentration of the second component.

After measuring the optical densities of the prepared solutions of the isomolar series (photocolorimeter KFK-3, the country of manufacture is Poland), plot the dependence of A on the ratio of concentrations or volumes of the components of the isomolar series and determine the position of the absorption maximum on the isomolar curve. The solution in which the content of the formed complex compound is the largest has the maximum light absorption. Therefore, the volume ratio of the components of the isomolar series corresponding to the absorption maximum corresponds to the stoichiometric ratio of the reactants.

If the absorption maximum on the isomolar curve is unclear, then its position is determined by extrapolation: straight lines are drawn through the initial points of both branches of the curve, extending them to the mutual intersection. The extrapolated point of intersection of the lines corresponds to the extreme point on the isomolar curve.

The optimal composition of the mixture, which will ensure maximum complexation of the selected hydrocolloids with histamine, was determined using the methodology of isomolar series and molar ratios.

3. Results and Discussion

According to the method of isomolar series, solutions of two components (low-esterified pectin substances and sodium alginate) and histamine of the same molar concentration ($1 \cdot 10^{-4}$ mol/dm³) were prepared, which were then mixed in ratios (from 1:9 to 9:1), keeping the total volume of the solution is unchanged. At the same time, the total number of moles of the structuring agent and histamine in the solution always remained constant. The experiment was carried out as follows: solutions of hydrocolloids and HisA with concentrations of $1 \cdot 10^{-4}$ mol/dm³ were prepared. 2 cm³ of a certain hydrocolloid solution and from 0.5 to 8.0 cm³ of HisA were poured into 10 volumetric flasks. Then the total volume of the mixture was brought up to 10 cm³ with water. After 1 hour, the optical density of the obtained solutions was measured and the saturation curve $\Delta A = f([HisA]/[hydrocolloid])$ was constructed. The breaking point on the saturation curve corresponds to the ratio of stoichiometric coefficients, which is equal to the ratio of the concentrations of the reacting components in the abscissa of the equivalence point.

After 1 hour, the optical density of the solutions was measured in the full UV spectrum. The comparison cuvette was filled with an aqueous solution of the appropriate hydrocolloid of a certain concentration – 10^{-4} mol/dm³.

Fig. 1 shows the comparative UV spectrum of solutions of NPR, AlgNa and a mixture of NPR+AlgNa hydrocolloids with histamine (HisA) at a concentration of $1 \cdot 10^{-4}$ mol/dm³ in the range of 255–305 nm in order to identify maxima.

According to the obtained data, the maximum for NPR is 265 nm, for AlgNa and the mixture of NPR-AlgNa hydrocolloids, it is 295 nm and 274 nm, respectively. These changes in the spectra, according to [35, 36], can be explained by the formation of the corresponding complexes.

The dependence graph (isomolar diagram) of the change in optical density from the ratio of component concentrations and the position of the absorption maximum on the obtained curve allows determining the composition of the complex compound by the extreme point on the isomolar diagram (Fig. 2–7).

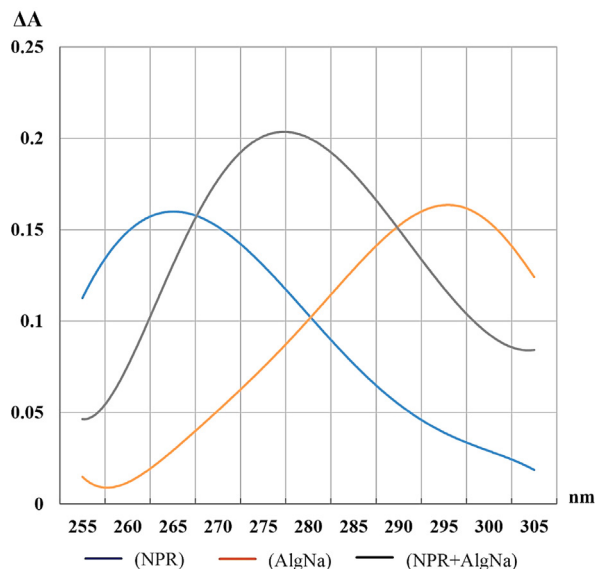


Fig. 1. Comparative UV spectrum of NPR, AlgNa solutions and a mixture of NPR-AlgNa+HisA hydrocolloids

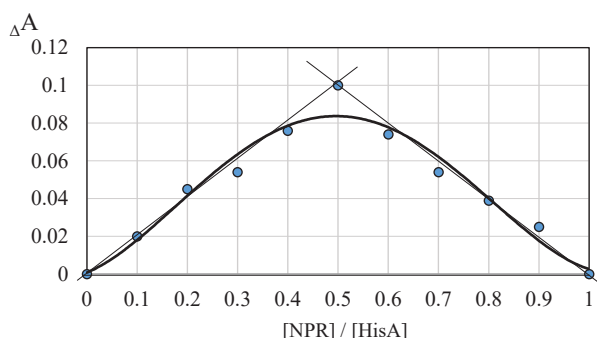


Fig. 2. Dependence of the change in optical density (ΔA) on the composition of the isomolar solution for NPR+HisA, $\lambda=265$ nm, $T=5$ °C

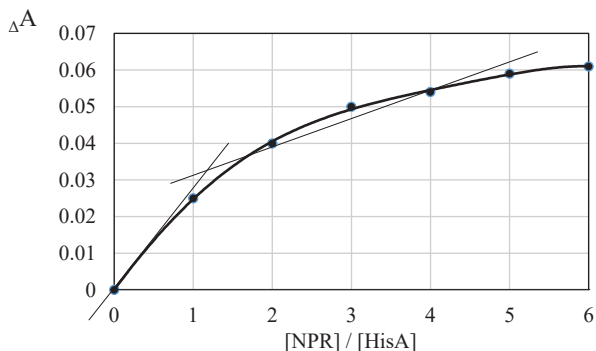


Fig. 3. Saturation curve for NPR+HisA, $\lambda=265$ nm, $T=5$ °C

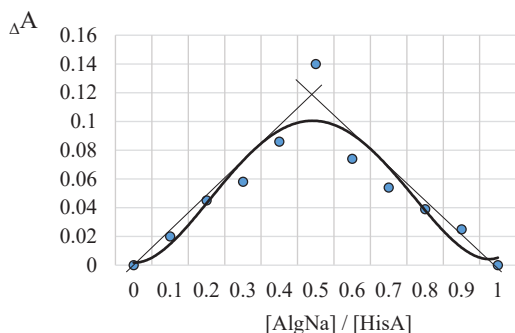


Fig. 4. Dependence of the change in optical density (ΔA) on the composition of the isomolar solution for AlgNa+HisA, $\lambda=295$ nm, $T=5$ °C

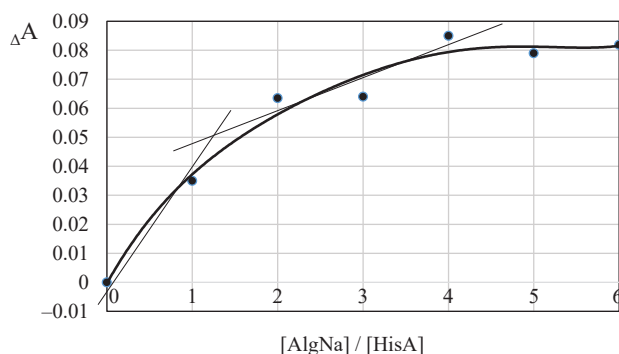


Fig. 5. Saturation curve for AlgNa+HisA, $\lambda=295$ nm, $T=5$ °C

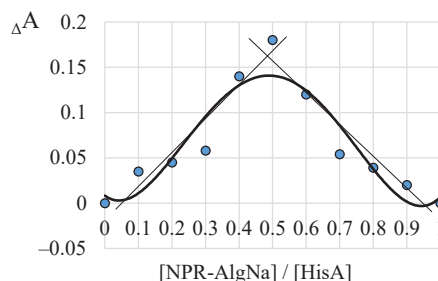


Fig. 6. Dependence of the change in optical density (ΔA) on the composition of the isomolar solution for NPR-AlgNa+HisA, $\lambda=274$ nm, $T=5$ °C

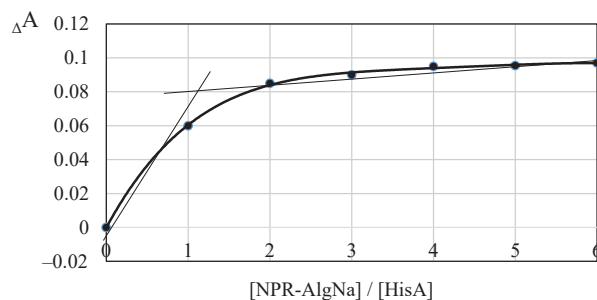


Fig. 7. Saturation curve for NPR-AlgNa+HisA, $\lambda=274$ nm, $T=5$ °C

Fig. 2, 4, 6 show the dependence of the change in optical density (ΔA) on the composition of the isomolar solution for NPR+HisA, AlgNa+HisA, NPR-AlgNa+HisA at the corresponding determined wavelength and $T=5$ °C. According to the obtained data, hydrocolloids form 1:1 complexes with HisA (one mole of HisA corresponds to one mole of hydrocolloid).

The choice of hydrocolloid for culinary products in gelatinous filling is based on the determination of the stability constant of the complexes.

The stability constant was calculated from the deviations of the experimental curve from tangents, as the tangent of the angle of inclination, on the saturation curves (Fig. 3, 5, 7). The saturation curves were obtained by the spectrophotometric method of isomolar series and molar ratios (measurements were performed for three parallel series, the confidence probability of the measurements was 96–98 %). The total concentration of components in the isomolar series was $1 \cdot 10^{-4}$ mol/dm³. In series of solutions with a constant concentration of HisA equal to $1 \cdot 10^{-4}$ mol/dm³, the concentration of hydrocolloids was changed from $0.25 \cdot 10^{-4}$ to $1.00 \cdot 10^{-4}$ mol/dm³.

Experimental data show that NPR, AlgNa, NPR-AlgNa form 1:1 complexes with histamine, which are characte-

rized by the following stability constants: $(3.07 \pm 0.5) \cdot 10^5$, $(3.72 \pm 0.5) \cdot 10^5$, $(3.94 \pm 0.5) \cdot 10^5$. The stability constant of HisA with NPR-AlgNa has higher values compared to other hydrocolloids.

Thus, the complex-forming ability of the studied hydrocolloids with respect to HisA was established. Each structural unit of hydrocolloids in the complex interacts with the HisA molecule in a 1:1 ratio.

In the further development of fillings based on hydrocolloids for fish preserves and culinary products, a mixture of NPR-AlgNa hydrocolloids was chosen.

The strengths of this study are that the determined ratio of hydrocolloids will allow to bind histamine as much as possible, and the products to which the composition of NPR-AlgNa will be added. It will also allow positioning it as products for a low-histamine diet, as well as a product with the additional functionality of preventive properties of dietary fibers, which is important for the HEALTHY-CAFE range.

The weaknesses include the use of sodium alginate biopolymer in these difficult conditions related to the logistics of imported food ingredients.

Additional opportunities provided that research results are used in the technology of health products in the HEALTHY-CAFE menu – increasing the competitiveness of the offered products due to the possibility of regulating the content of biogenic amines.

The developments proposed in this paper are of a practical nature, but require socio-economic justification for the formation of the foundations of the development of a low-histamine diet in HEALTHY-CAFE in Ukraine.

In the conditions of martial law in Ukraine, it is not up to the rules of a balanced diet. People eat what is available, so HEALTHY-CAFE can help unobtrusively establish this balance through a menu of wellness referrals.

4. Conclusions

The study of the comparative UV spectrum of solutions of NPR, AlgNa and a mixture of NPR+AlgNa hydrocolloids with histamine, obtained according to the method of isomolar series, made it possible to determine the maxima that were for NPR at 265 nm, for AlgNa and a mixture of NPR-AlgNa hydrocolloids at 295 nm and 274 nm, respectively. These maxima in the spectra can be explained by the formation of the corresponding complexes.

The isomolar diagram constructed for each of the investigated variants of the formed complexes allows to determine the composition of the complex compound by the extreme point. The stability constants of the formed NPR, AlgNa, NPR-AlgNa complexes with histamine were determined, which confirms the complex-forming ability of the studied hydrocolloids in relation to biogenic amines.

The study of the change in optical density (ΔA) from the composition of an isomolar solution for NPR+HisA, AlgNa+HisA, NPR-AlgNa+HisA at the corresponding determined wavelength allows to determine the composition of the composition of hydrocolloids, which corresponds to the maximum complexing ability. Each structural unit of hydrocolloids in the complex interacts with the HisA molecule in a 1:1 ratio. Such results allow to conclude about the expediency of using the composition of hydrocolloids in the technology of fish products for a low-histamine diet in HEALTHY-CAFE.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

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Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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