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Development of an iron-based oxygen-scavenging nanofilm to extend the shelf-life of ultrafiltered cheese

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ABSTRACT

Oxygen is one of the main factors contributing to food spoilage. A recent approach in food packaging is the use of oxygen scavengers within the package to prevent quality deterioration in oxygen-sensitive foods. Removing oxygen inside the package by a scavenger, along with low gas impermeability of the packaging film, can prevent the growth of molds and yeasts in the food and increase its shelf life. For this purpose, in previous research, to increase the shelf life of ultrafiltered cheese, new nanocomposites based on polyolefin elastomer containing nanoparticles (nanosilica and nanographene) with an iron-based oxygen scavenger were prepared and optimized using the D-optimal mixture design method. Based on this design, various treatments were obtained with varying concentrations of different nanoparticles in the constant components of polyolefin elastomer and POE-g-MAH compatibilizer. Then, the best base film was obtained based on oxygen permeability, oxygen absorption, and mechanical properties at a concentration of 0.33% iron, 0.21% graphene, and 0.46% silica with a desirability of 76.2%. In this study, the effect of the optimal nanocomposite film on the microbial, chemical, and sensory characteristics of ultrafiltered cheese was investigated. The results showed that using this scavenger film in the ultrafiltered cheese package, while preserving its sensory characteristics, was able to extend the shelf life of the cheese compared to the control sample. It can be concluded that this new nanofilm, as an oxygen scavenger, has suitable efficiency for increasing the shelf life of oxygen-sensitive products.

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1. Introduction

Cheese is a nutritious, diverse, and valuable dairy product consumed widely worldwide. In Iran, one of the most popular types is a relatively high-fat fresh cheese produced using the ultrafiltration (UF) method (1). However, the growth of mold and yeast in UF cheese is one of the main reasons for its low shelf life and high return rates. Important goals such as increasing product diversity, reducing contamination,

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increasing shelf life, enhancing sales appeal, and preserving cheese quality can be achieved by applying modern packaging methods (2).

Oxygen is the main cause of cheese spoilage because it promotes the growth of aerobic microorganisms, oxidation of cheese components, loss of nutritional value, development of off-flavors, unacceptable color changes, shorter shelf life, and decreased food safety. Therefore, controlling the oxygen level in the packaging of sensitive products such as cheese is essential for maintaining product quality and extending its shelf life. Using gas mixtures with reduced oxygen, can prolong the shelf life of cheeses, especially semi-hard and hard cheeses (3). Modified atmosphere packaging, vacuum packaging, and oxygen scavengers are current alternatives used to reduce or to eliminate oxygen in food packaging (4). However, both MAP and vacuum packaging require expensive equipment for cheese packaging and do not ensure complete oxygen removal (5). A more recent approach in food packaging involves using oxygen scavengers within the package to prevent quality deterioration in oxygen-sensitive foods. Oxygen scavengers are an effective solution for removing oxygen permeating through the packaging film and for overcoming the limitation of in MAP and vacuum packaging. Oxygen scavengers were first introduced to the market in the late 1970s by the Japanese company Mitsubishi Gas Chemical under the name Ageless®. These scavengers can remove oxygen from the package headspace and even oxygen that permeates through the packaging material during storage (6). Many commercial oxygen scavengers available in the market contain iron particles in the form of small sachets to prevent changes in the product's color, odor, and taste (7). These sachets are designed to reduce the oxygen level to less than 1% in the package (8). Considering the risk of misuse or consumption of these sachets by consumers, especially by children, and the risk of product contamination by the sachet make the incorporation of scavengers directly into single-layer or multilayer packaging a safer and more acceptable option. Additionally, due to the need for extra steps to add sachets in the packaging process and their inapplicability for some foods and beverages, incorporating scavengers into the packaging structure itself lowers potential costs, increases efficiency, and facilitates production (9). Thus, recent research in active packaging has focused on using these scavengers into packaging materials (10). Using iron nanoparticle-based oxygen scavengers directly in the packaging film structure or incorporating them into different layers of a multilayer packaging film can be considered a replacement for sachets in food packaging (11).

One notable characteristic that determines the expected product shelf life is the gas permeability property of the polymer used in food packaging (12). The theory regarding these nanocomposites is that the homogeneous dispersion of nano-fillers in the polymer matrix can affect the permeability characteristics of the polymer film. In fact, nanoparticles create a tortuous path for permeating substances, and substances must pass around these nanoparticles instead of taking a direct path perpendicular to the film surface. As a result, the pathway becomes longer, which reduces the rate of gas transmission through the material (13). In addition, incorporating nano-fillers into polymer blend matrices can improve other properties such as

mechanical, thermal, electrical, and optical characteristics (14).

In our previous study, polyolefin elastomer (POE), a relatively new type of copolymer, was used as the polymer matrix in the development of our nanocomposite film (15). POE offers many advantages as a polymer, but certain drawbacks, such as its weak barrier properties, have limited its acceptance in the consumer market (16). To address this issue, silica nanoparticles were incorporated due to their homogeneous dispersion within polymer matrices and their ability to improve various properties, including reduced permeability to gases such as water vapor and oxygen, as well as enhanced mechanical strength (17). Graphene was also used because of its unique characteristics, including a high surface area and excellent mechanical, thermal, and optical properties (18), along with its strong compatibility with polymer matrices. Graphene nanoparticles can significantly increase the glass transition temperature of nanocomposites through strong intermolecular interactions with the polymer matrix (19). A well-dispersed and orderly distribution of graphene nanoparticles within the polymer matrix improves the tensile strength and oxygen barrier properties of the nanocomposite. Moreover, these nanoparticles exhibit strong antimicrobial activity by inhibiting bacterial growth through cell wall disruption (20).

Therefore, eliminating oxygen inside the packaging through oxygen absorbers, along with using an oxygen-impermeable film for ultrafiltered (UF) cheese packaging, can prevent the growth of molds and yeasts and extend the product's shelf life. In this study, the potential for removing headspace oxygen using a designed oxygen-absorbing film was investigated to enhance the shelf life of ultrafiltered cheeses.

2. Materials and Methods

2.1. Materials

All materials used in this study, including polyolefin elastomer (POE), POE-g-MAH compatibilizer, nanosilica, iron nanoparticles, and graphene, were identical to those described in our previous work (21).

2.2. Production of active nanocomposite film

The preparation of the active nanocomposite film has been previously described in detail (21). Briefly, POE, POE-g-MAH, nanosilica, iron nanoparticles, and graphene were mixed according to the optimized formulation obtained from a D-optimal mixture design. The dried components were compounded using a Werner & P fleiderer ZSK25 co-rotating twin-screw extruder at 130–170°C and 150 rpm, pelletized, and compression-molded into films (1 mm thickness) at 170°C and 20 MPa. The optimized film was laminated onto aluminum foil to form oxygen scavenger lids for UF cheese packaging, which were used in the current microbial and sensory evaluations.

2.3. Statistical analysis and experimental design

The optimization process for selecting the best film formulation was carried out using the D-optimal mixture design approach as detailed in our previous publication (21). In this study, the optimal film identified in that work was applied as an active packaging material for UF cheese, and its effect on microbial growth, pH, acidity, and sensory attributes was evaluated during storage.

2.4. Proteolysis evaluation

Soluble nitrogen at pH 4.6 was extracted using the method described by Sousa and colleagues. Twenty grams of ground and homogenized cheese sample were weighed into zip-lock bags, mixed with 40 mL of distilled water in a stomacher, and transferred into centrifuge tubes. The pH of the samples was adjusted to 4.6 using 1 N hydrochloric acid and, after 30 min at room temperature, readjusted to 4.6. The tubes were then incubated in a water bath at 40°C for 1 h. Following incubation, the samples were centrifuged in a refrigerated centrifuge at 4°C and 4290 rpm. The supernatant was filtered through Whatman No. 1 paper, and the nitrogen content of 9 mL of the filtered liquid was determined using the Kjeldahl method.

2.5. Lipolysis evaluation

Fat from cheese samples was extracted using diethyl ether, and the acid value of the fat was determined by titration with alcoholic potassium hydroxide. Five grams of cheese sample were thoroughly ground with three grams of anhydrous sodium sulfate, placed in capped glass containers, and mixed with 30 mL of diethyl ether using a magnetic stirrer for 1 h. The mixture was then filtered through Whatman No. 1 paper, and the residue remaining in the container was washed three times with 10 mL portions of diethyl ether. Five drops of phenolphthalein were added to the collected solvent, which was then titrated with alcoholic potassium hydroxide until a pale pink color appeared. After titration, the solvent was evaporated under vacuum, and the remaining fat was weighed to determine the total fatty acid content.

2.6. Total microorganism count

The total microbial count was performed according to National Standard No. 5784 using the pour plate method on Plate Count Agar with incubation at 37°C for 48 h.

2.7. Starter bacteria count

For counting starter bacteria, 5 g of cheese were transferred into a stomacher bag using a sterile knife, and 45 mL of sterile 0.1% peptone water was added. The sample was homogenized in a stomacher at 260 rpm for 2 min. Serial dilutions were prepared by adding 1 mL of each dilution to 9 mL of sterile 0.1% peptone water. The desired dilution was plated on M17 agar using the pour plate method, and plates were incubated at 37°C for 72 h.

2.8. Total coliform count

Total coliforms were determined according to the Microbiological Standards for Milk and Dairy Products No. 2406. The pour plate method was used with Violet Red Bile Agar (VRB, Merck, Germany). Plates were incubated at 30°C for 24 h, and results were recorded in g/cfu using a colony counter.

2.9. Mold and yeast count

Mold and yeast counts were also performed according to Standard No. 2406. Samples were cultured using the surface plating method on YGC agar (Merck, Germany) and incubated at 25°C for 5 days. Colonies were counted with a colony counter and reported in g/cfu.

2.10. Titratable acidity and pH measurement

Titratable acidity was measured according to National Standard No. 2852 and expressed as lactic acid. For titratable acidity, 10 g of cheese were mixed with 40 mL of distilled water and titrated with 0.1 N NaOH in the presence of 0.5 mL phenolphthalein. pH was

measured at room temperature using a pH meter following the same standard.

2.11. Moisture and dry matter

Moisture and dry matter were determined according to National Standard No. 1753. Five grams of sample were placed in a pre-weighed container and dried in an autoclave at 100°C until a constant weight was reached. The weight loss represented moisture and volatile substances, while the remaining weight corresponded to dry matter.

2.12. Sensory evaluation

Sensory evaluation was conducted using a 5-point hedonic scale. Ten PhD students in Food Science and Technology were selected as panelists. Cheese samples were assessed for taste, odor, color, texture, and overall acceptance at the Faculty of Nutrition and Food Science, Shahid Beheshti University. Scores ranged from 5 for excellent to 1 for very poor.

3. Results

3.1. Total bacterial count

The microbiological characteristics of the different cheese samples, evaluated in three replicates, are presented in Table 1. The total microorganism counts for the two UF cheese treatments during storage on days 1, 15, 30, 45, and 60 are also shown in Table 1. The results indicate that the total aerobic bacterial count in both the control and optimized treatments decreased during storage up to day 45. This reduction was significantly greater in the cheese packed with the optimized film compared to the control, with the bacterial count reaching 4.23 log cfu/g in the optimized sample and 6.14 log cfu/g in the control sample at the end of the storage period.

Bacterial counts in both treatments remained below the Iranian National Standard No. 2406 limit (1000 cfu/g) throughout the storage period, but counts in the control treatment were significantly higher than in the optimized treatment.

3.2. Starter bacteria count

The changes in starter bacteria count showed an increase from 5.7 log cfu/g to 8.7 log cfu/g in the control treatment and from 5.6 log cfu/g to 6.9 log cfu/g in the optimized film treatment during storage. Starter bacteria counts in the control were significantly higher than in the optimized treatment at all times.

3.3. Mold and yeast count

Mold and yeast counts in the treatments indicated that in the optimized film treatment, they remained below the detectable limit until day 45 and reached only 1.36 cfu/g at the end of storage. In contrast, counts in the control increased significantly during storage, reaching 5.88 cfu/g. Fungi, particularly some species of *Penicillium* and *Aspergillus*, are major contaminants in dairy products due to their production of mycotoxins, posing public health risks. In this study, mold and yeast counts in the control increased significantly from 2.23 cfu/g on day 30 to 5.88 cfu/g on day 60 under refrigerated storage, whereas in the optimized film treatment, counts remained below detectable limits for 45 days and reached only 1.36 cfu/g at the end of the storage period.

3.4. Total coliform count

Results showed that no coliform contamination was detected in either the control or the optimized film-treated cheese at any sampling time.

Table 1. Microbial results of samples (log cfu/g)

Parameter	Treatment	Day 1	Day 15	Day 30	Day 45	Day 60
Total count	Optimal	6.20±0.12 Aa	5.80±0.09 Bb	4.64±0.05 Bc	4.10±0.03 Bd	4.23±0.05 Bd
	Control	6.23±0.16 Ab	6.58±0.11 Aa	5.48±0.09 Ac	5.76±0.10 Ac	6.14±0.08 Ab
Mold & yeast count	Optimal	N.D	N.D	N.D	N.D	0.07±1.36 A
	Control	N.D	N.D	2.23±0.09 Ac	3.84±0.12 Ab	5.88±0.14 Aa
Starter count	Optimal	5.6±0.05 Ac	5.9±0.04 Bbc	6.4±0.05 Bab	6.6±0.09 Ba	6.9±0.07 Ba
	Control	5.7±0.02 Ae	6.6±0.04 Ad	7.3±0.08 Ac	7.5±0.10 Ab	7.8±0.03 Aa

*Different uppercase and lowercase letters indicate significant differences ($p<0.05$) in similar columns between the two treatments and in rows among storage days, respectively.

Table 2. Lipolysis and proteolysis results of treatments

Treatment	Proteolysis (% w/w)			Lipolysis (0.01 mLKOH)		
	Day 3	Day 30	Day 60	Day 3	Day 30	Day 60
Optimal	0.01±0.38 Aa	0.03±0.40 Aa	0.04±0.41 Ba	2±18 Ab	3±28 Bb	8±59 Ba
Control	0.02±0.37 Ab	0.39±0.01 Ab	0.06±0.47 Aa	4±22 Ac	5±42 Ab	7±71 Aa

* Different uppercase and lowercase letters indicate significant differences ($p<0.05$) in similar columns between the two treatments and in rows among storage days, respectively.

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Table 3. Chemical analysis results in cheese samples during storage period

Day	pH		Acidity		Moisture (% w/w)		Dry Matter (% w/w)	
	Control	With OS	Control	With OS	Control	With OS	Control	With OS
1	4.87±0.02 Aa	4.83±0.03 Aa	1.42±0.01 Bc	1.46±0.03 Ac	63.82±0.09 Aa	62.86±0.05 Ba	36.52±0.10 Be	37.68±0.07 Ab
15	4.79±0.04 Bb	4.76±0.05 Bb	1.47±0.03 Abc	1.48±0.04 Aab	62.57±0.06 Ab	62.41±0.03 Aa	37.38±0.07 Bd	37.81±0.02 Ab
30	4.71±0.01 Ac	4.72±0.02 Acd	1.51±0.05 Aab	1.49±0.01 Aab	62.76±0.12 Ac	62.85±0.07 Aa	37.56±0.04 Bc	37.76±0.05 Aab
45	4.65±0.02 Bcd	4.70±0.01 Ad	1.55±0.04 Aa	1.51±0.02 Ba	61.42±0.06 Bd	62.79±0.06 Aa	37.89±0.05 Bb	37.85±0.01 Ab
60	4.69±0.03 Ad	4.71±0.03 Acd	1.52±0.07 Aab	1.50±0.03 Aab	60.23±0.13 Be	61.91±0.03 Bb	38.57±0.09 Aa	38.16±0.12 Ba

*Different uppercase and lowercase letters indicate significant differences ($p<0.05$) in rows between the two treatments and in columns among storage days, respectively.

3.5. Proteolysis

The results of proteolysis and lipolysis for the treatments are presented in Table 3. Proteolysis results showed that at the end of the storage period, the control sample had a significantly higher degree of proteolysis compared to the optimized sample. No significant difference was observed between the two treatments up to day 30.

3.6. Lipolysis

As shown in Table 3, the free fatty acid content resulting from lipolysis was significantly higher in the control sample compared to the optimized sample throughout the storage period. Free fatty acid content increased from 22 mL of 0.01 M KOH at the start of storage to 71 mL of 0.01 M KOH at the end.

3.7. Chemical properties

The results of pH, titratable acidity, moisture, and dry matter are presented in Table 3. Acidity increased significantly in both optimized and control samples up to day 45, but then decreased significantly in the control sample. No significant difference in acidity between the control and optimized treatments was observed throughout storage. pH values decreased over time in both treatments, with a slight increase in the control at the end of storage. The control sample's pH ranged from 4.87 to 4.69, and the optimized sample from 4.83 to 4.71, with no significant differences at any time point. The reported results in Table 3 show that the pH value in both control and optimal treatments decreased significantly during the storage period. At the end of storage, pH reached 4.69 in the control and 4.71 in the

optimized sample, with no statistically significant difference.

Moisture content in the control decreased from 63.82% on day 3 to 60.23% at the end of storage, whereas the optimized sample with the oxygen-absorbing film showed no significant changes during storage except for a decrease at the end. Dry matter content in the control increased significantly from 36.52% to 38.57%, while in the optimized sample, it remained stable except at the end of storage. As shown in Table 3, the control sample exhibited a significant decrease in moisture and an increase in dry matter over time, whereas the optimized nanocomposite film with oxygen absorber, maintained moisture and dry matter levels more effectively.

3.8. Sensory evaluation

The sensory characteristics of UF cheese samples without and with oxygen absorbers on days 30 and 60 are presented in Fig 1 and 2. According to the sensory evaluation results, there was no significant difference in taste between the treatments on day 30. However, on day 60, the control sample scored significantly higher in taste. No significant differences were observed between samples in terms of odor and color throughout the storage period. In contrast, texture and consistency received significantly higher scores for the sample containing the oxygen absorber compared to the control on both days. No significant differences were found in acceptance scores between the two samples on days 30 and 60. In this study, the sensory properties of Feta cheese were evaluated, and no adverse effects on color, odor, taste, or texture were observed in the samples containing the oxygen-absorbing film. The texture and consistency of cheeses with the oxygen absorber were more acceptable than the control throughout storage.

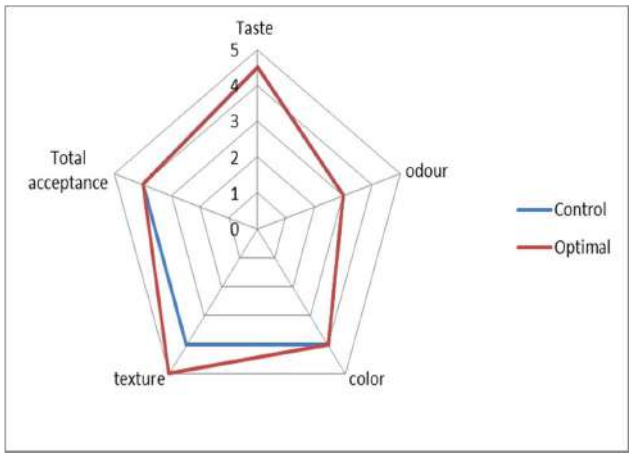

Figure 1. Sensory evaluation results of cheese samples on day 30

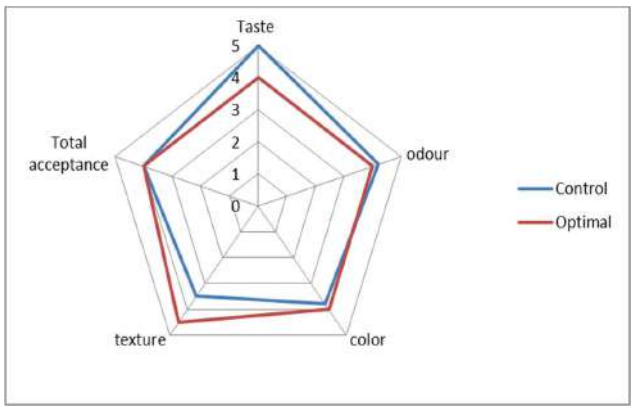

Figure 2. Sensory evaluation results of cheese samples on day 60

4. Discussion

4.1. Total and starter bacterial counts

The optimized film, by reducing oxygen in the package headspace, was able to limit bacterial growth and better preserve cheese quality compared to the control. Since starter bacteria are facultative anaerobes, the aerobic conditions in the control sample promoted their growth and increased bacterial cell yield (22). Dermiki et al. (2008) similarly found that cheeses packaged under vacuum or modified atmosphere (CO_2 presence, absent oxygen) reached allowable bacterial limits much later than air-packaged controls (23). Whitley et al. (2000) also reported reductions in starter bacteria in modified atmosphere packaging, attributing it to high CO_2 and lack of oxygen (24).

4.2. Mold and yeast spoilage

Fungi, particularly *Penicillium* and *Aspergillus* species, pose public health risks due to mycotoxin production. The significant difference in mold and yeast counts demonstrates that the optimized film played a critical role in inhibiting fungal growth by absorbing oxygen. These findings align with Dermiki et al. (2008) and Panfil-Kuncewicz et al. (2015), who showed that low oxygen environments (via MAP, vacuum, or ATCO oxygen absorbers) effectively suppress mold and yeast (3, 22). Fungal spoilage in UF cheeses is rarely due to pasteurization failures; rather, it is often caused by airborne contamination in the factory, particularly in the cheese coagulation tunnel, or from packaging materials (25).

4.3. Proteolysis and lipolysis

The higher degree of proteolysis in the control sample was primarily due to the increased growth of mesophilic and psychrotrophic bacteria, whose proteases and peptidases break down proteins (26). Lactic acid bacteria also contribute via extensive proteinase systems (27). This matches Gonzalez-Fandos et al. (2000), who found higher nitrogen compound levels in air-packaged control cheeses (28). The increase in free fatty acids (an indicator of lipolysis) is mainly due to microbial lipase activity breaking down lipids. Robertson (2014) similarly indicated that vacuum packaging effectively reduced lipolysis (29), though Pintado and Malacta (2000) found no significant atmosphere-related differences (30).

4.4. Chemical properties

The expected decrease in pH over storage is driven by lactose fermentation by starter bacteria into lactic acid, and the production of amino acids and free fatty acids (31). The slightly lower pH in the control is likely due to greater lactic acid production under aerobic conditions, while a late-stage slight pH increase may reflect proteolytic ammonia production or lactic acid breakdown (32). Overall, acid production was faster in the control due to higher bacterial counts.

Moisture is a critical factor affecting cheese texture and rheology. The control sample exhibited significant moisture loss and dry matter increase due to higher gas permeability in its packaging. Conversely, the optimized nanocomposite film, utilizing well-dispersed nanoparticles (graphene platelets) in the polymer matrix, inhibited moisture migration and preserved cheese hydration (32, 33).

4.5. Sensory properties

Sensory attributes drive consumer acceptance (34). The oxygen-absorbing film successfully maintained acceptable color, odor, and taste while significantly improving texture and consistency. This textural

improvement is directly linked to the moisture barrier properties of the film, which retained hydration in the optimized sample (33, 35). The slightly higher taste score of the control on day 60 was likely due to increased lipolysis; the resulting free fatty acids convert into methyl ketones and thioesters, which enhance flavor and aroma. Ultimately, the lack of difference in overall acceptance indicates that utilizing the oxygen-absorbing film does not negatively impact consumer perception.

5. Conclusion

The optimized nanocomposite film significantly inhibited mold and yeast growth in UF cheese by actively scavenging oxygen. Moreover, its application successfully preserved the cheese's sensory qualities and extended its shelf life compared to the control. These results demonstrate that this novel nanofilm is an effective oxygen-scavenging solution for enhancing the shelf life of oxygen-sensitive products.

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Authorship contribution

Zhaleh Sheidaei: Conceptualization of idea, resources, visualization, data curation, writing of original draft, writing of review and references.

Mahmood Alizadeh Sani: Conceptualization, supervision, data curation, formal analysis, validation, reviewing, and editing.

Mehdi Farhoodi: Methodology, software, validation, data curation, review and editing.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Data availability

All necessary data has been duly supplied in this study.

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