

## **A HUMIC ACID–LACCASE SYSTEM AS A TUNABLE COLORIMETRIC PLATFORM FOR FOOD PACKAGING INTEGRITY INDICATORS**

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This work was supported by the Russian Science Foundation (RSF) under grant №24-76-10093, «Development of optically active indicators for smart food packaging».

### **Abstract**

The development of novel methods for monitoring the integrity of food packaging is an important task for the food industry. The study presents a colorimetric platform based on the enzyme-substrate system for detecting oxygen leaks in packaging materials. The system is based on the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by laccase to form the colored radical cation ABTS<sup>•+</sup>. Humic acids (HAs) act as natural modulators. The reaction does not occur in an oxygen-free environment, which ensures the indicator remains unresponsive in modified atmosphere packaging (MAP). Control experiments confirmed that the oxidation of ABTS in a solution with HAs does not occur without the addition of an enzyme. It was found that an increase in the concentration of HAs from 0 to 0.05 % reduces the initial reaction rate by 97 %. This is due to the dual function of HAs, which act as competitive inhibitors of laccase, as well as reductants of the radical ABTS<sup>•+</sup>. The color response and response time of the system are determined by the concentration of the modulator – from green (minutes) to brown (24 hours). The use of natural components ensures environmental safety, and the ability to control the kinetics of the reaction allows the indicator to be adapted to various products and storage conditions. The obtained results demonstrate a great opportunity of the system as a colorimetric platform with a tunable response time.

### **Keywords**

Humic acids, laccase, integrity indicator, colorimetric sensor

## **СИСТЕМА НА ОСНОВЕ ГУМИНОВАЯ КИСЛОТА–ЛАККАЗА КАК РЕГУЛИРУЕМАЯ КОЛОРИМЕТРИЧЕСКАЯ ПЛАТФОРМА ДЛЯ СОЗДАНИЯ ИНДИКАТОРОВ ЦЕЛОСТНОСТИ ПИЩЕВОЙ УПАКОВКИ**

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This work was supported by the Russian Science Foundation (RSF) under grant №24-76-10093, «Development of optically active indicators for smart food packaging».

### **Аннотация**

Разработка новых методов контроля целостности упаковки продуктов является важной задачей для пищевой промышленности. В работе представлена колориметрическая платформа на основе фермент-субстратной системы для отслеживания герметичности упаковочных материалов. В основе системы лежит реакция окисления 2,2'-азино-бис(3-этилбензтиазолин-6-сульфоукислоты) (ABTS) лакказой с образованием окрашенного катион-радикала ABTS<sup>•+</sup>. Гуминовые кислоты (ГК) выступают в качестве природных модуляторов. В бескислородной среде реакция не протекает, что обеспечивает стабильность индикатора в упаковке с модифицированной атмосферой (МАР). Контрольные эксперименты подтвердили, что окисление ABTS в растворе с

ГК не происходит без добавления фермента. Установлено, что увеличение концентрации ГК от 0 до 0,05 % снижает начальную скорость реакции на 97 %. Это обусловлено двойной функцией ГК, которые действуют как конкурентные ингибиторы лакказы, а также как восстановители радикала ABTS<sup>•+</sup>. Цветовой отклик и время срабатывания системы определяются концентрацией модулятора – от зеленого (минуты) до коричневого (24 часа). Использование натуральных компонентов обеспечивает экологическую безопасность, а возможность контролировать кинетику реакции позволяет адаптировать индикатор к различным продуктам и условиям хранения. Полученные результаты демонстрируют перспективность системы в качестве колориметрической платформы с регулируемым временем срабатывания.

#### **Ключевые слова**

Гуминовые кислоты, лакказы, индикатор целостности, колориметрический сенсор

Oxygen detection is an important analytical task. Optical sensors for this purpose are attracting increasing interest, especially in the field of MAP [1]. Traditional indicators are usually synthetic products that do not always meet modern safety and environmental requirements [2]. In this regard, the development of new colorimetric systems based on natural components is an urgent task. Among the natural compounds capable of modulating enzymatic reactions, HAs are of particular interest. Due to the combination of antioxidant properties and the ability to interact with oxidoreductases, HAs can serve as natural modulators of enzymatic reactions. This makes them promise for the development of food packaging integrity indicators.

One of the main causes of food spoilage is oxygen, that is why MAP is used [3]. The laccase-ABTS system has great potential as an oxygen-sensitive indicator, as the enzyme requires oxygen to start the reaction. ABTS is converted into green cation radical ABTS<sup>•+</sup>. However, the unmodified reaction proceeds quickly, which may not be optimal for controlling the gradual supply of oxygen over a long period of time. Therefore, regulation of reaction kinetics is important for practical applications. HAs are macromolecules of organic matter rich in phenolic groups, which allows laccase to integrate oxygen into them. The hypothesis that HAs can be effective modulators of the laccase-ABTS reaction was tested, which makes it possible to control the response time.

The oxidation of laccase-based ABTS was measured spectrophotometrically at 420 nm in acetate buffer (pH 4.31). The concentration of ABTS varied from 0.15 to 0.6 mM as HAs concentration increased from 0.014 to 0.05 %. The concentration of laccase in the solution was constant and equal to 0.03 mg/mL. Control experiments confirmed that in the absence of oxygen the reaction did not occur, regardless of the presence of HAs. The results demonstrate a clear dose-dependent modulation of the reaction rate. Thus, at 0.15 mM ABTS HAs reduced the initial reaction rate by 97 % (34.5 times). Similarly, at 0.3 mM ABTS, the rate decreased from 0.138 to 0.004  $\Delta$ Abs/min and at 0.6 mM ABTS from 0.134 to 0.010  $\Delta$ Abs/min under the same HAs concentrations. The inhibitory effect was less pronounced at the highest substrate concentration, which indicates a competitive type of enzyme inhibition.

The color response of the system directly depended on the degree of inhibition. At low concentrations of HAs (0.014 %), the green color of the radical ABTS<sup>•+</sup> prevailed, and the response time was about 5 minutes. As the HAs concentration increased, the green color gradually changed to brown, and this transition occurred within a few hours. At 0.05 % HAs the system remained stable for 24 hours before reaching the final brown color. Additional experiments with gel matrices demonstrated similar behavior. The HAs-laccase-ABTS system retains its tunable color characteristics in hydrogels. This experiment confirmed that the modulating effect of HAs is present in solid media. Changing the concentration of humic acids in such a colorimetric platform makes it possible to adapt the indicator to different products and storage conditions. Further work will focus on choosing a non-toxic laccase substrate for validation of the system. Moreover, the effectiveness of the HAs-laccase system will be tested under production-used food packaging conditions.

### **Literature**

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