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THE EVALUATION OF THE ANTIOXIDANT POTENTIAL DURING THE OXIDATIVE POLYMERIZATION OF POLYPHENOL COMPOUNDS INDUCED BY THE LACCASE ENZYME

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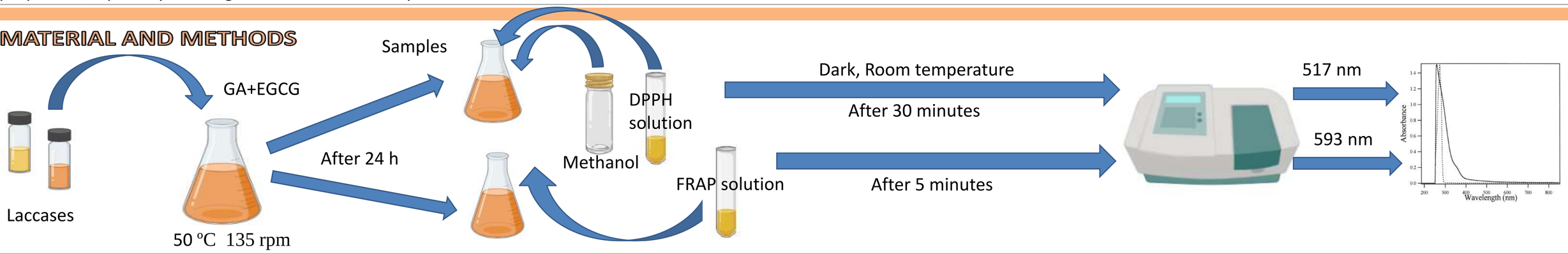
INTRODUCTION

Laccases are polyphenol oxidases belonging to the multi-copper protein's family. They have significant role in different industrial fields, especially in food industry (stabilization of beer, wine, juices and baking) due to their ability to catalyze oxidative polymerization of wide range of various phenol and non-phenol compounds. Polyphenols are main class of secondary metabolites present in many species of vegetables and fruits, that have high antioxidant activity. Most of vegetables and fruits are consumed after different chemical and physical processes that change characteristics and quality of food, affecting changes in quantity and quality of bioactive compounds, phenols. These processes can cause reduction of antioxidant activity of phenols and harmful effects on human health. Because of that, the oxidative polymerization of polyphenols by laccases have important role in the stabilization of different food products. Moreover, this process is more environmentally friendly and economically viable than chemical and physical processes. The gallic acid (GA) and epigallocatechin gallate (EGCG) are two polyphenols present in different plants with high antioxidant activity. Thus, this study investigates the oxidative polymerization potential of GA and EGCG mixture with laccases from white rot fungi (WRF) and commercial one in order to obtain products with satisfactory properties, especially with higher antioxidant activity.

MATERIAL AND METHODS



RESULTS

The maximal antioxidant activity, measured by both DPPH method (inhibition of 58.58% of radicals) and FRAP method (176.57 mmol Fe^{2+}/ml) was achieved by a sample containing a mixture of polyphenols and 0.3 U/ml laccase enzymes, after 14 h, at 50˚C (Fig. 1, Table 1).

Table 1. Antioxidant activity of control and samples determined by DPPH and FRAP methods after 24h incubation (50 °C).

Samples	DPPH, % inhibition	FRAP, mmol Fe^{2+}/l
Control (GA+EGCG)	30.43	123.04
GA+EGCG +Commercial laccase	57.69	167.01
GA+EGCG +*WRF* laccase	57.01	162.81

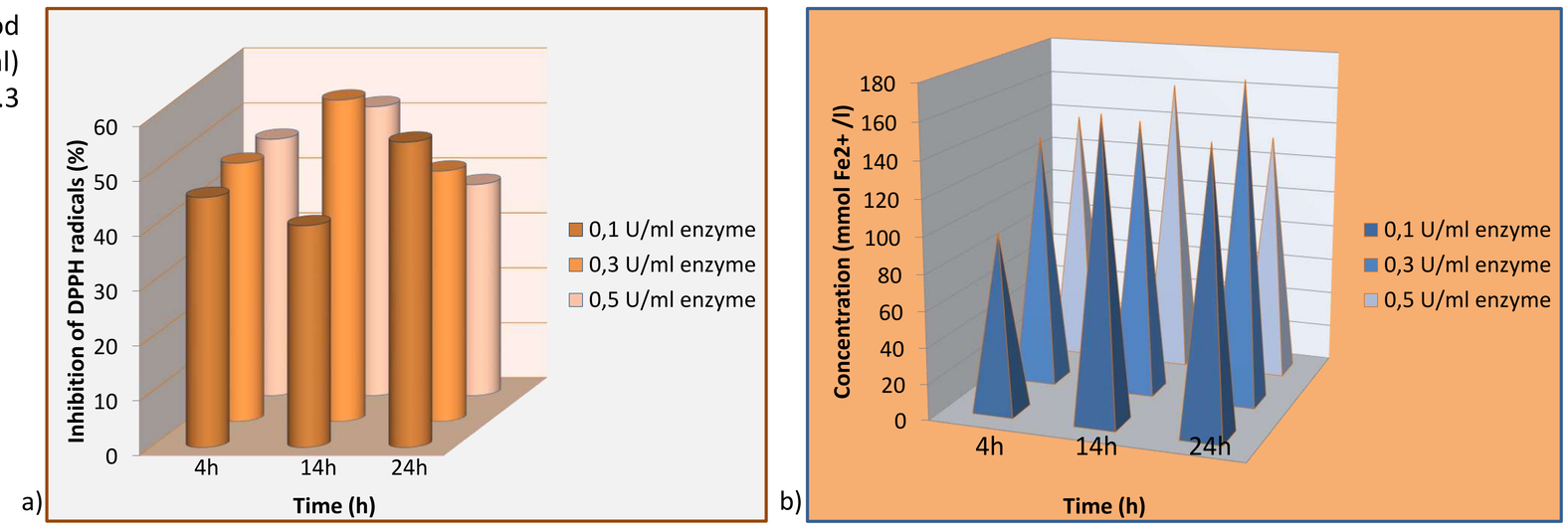


Figure 1. Optimization of polyphenol polymerization of GA + EGCG + Commercial laccase: DPPH method (a), FRAP method (b)

The difference in color intensity of the samples potentially indicates the degree of polyphenols polymerization that occurred in the samples after different incubation time. The lightest samples were incubated for 4 h, the dark samples for 14 h and the darkest for 24 h (Fig. 2).

Figure 2. Qualitative analysis of polymerization of sample GA+EGCG+Commercial laccase with different time of incubation

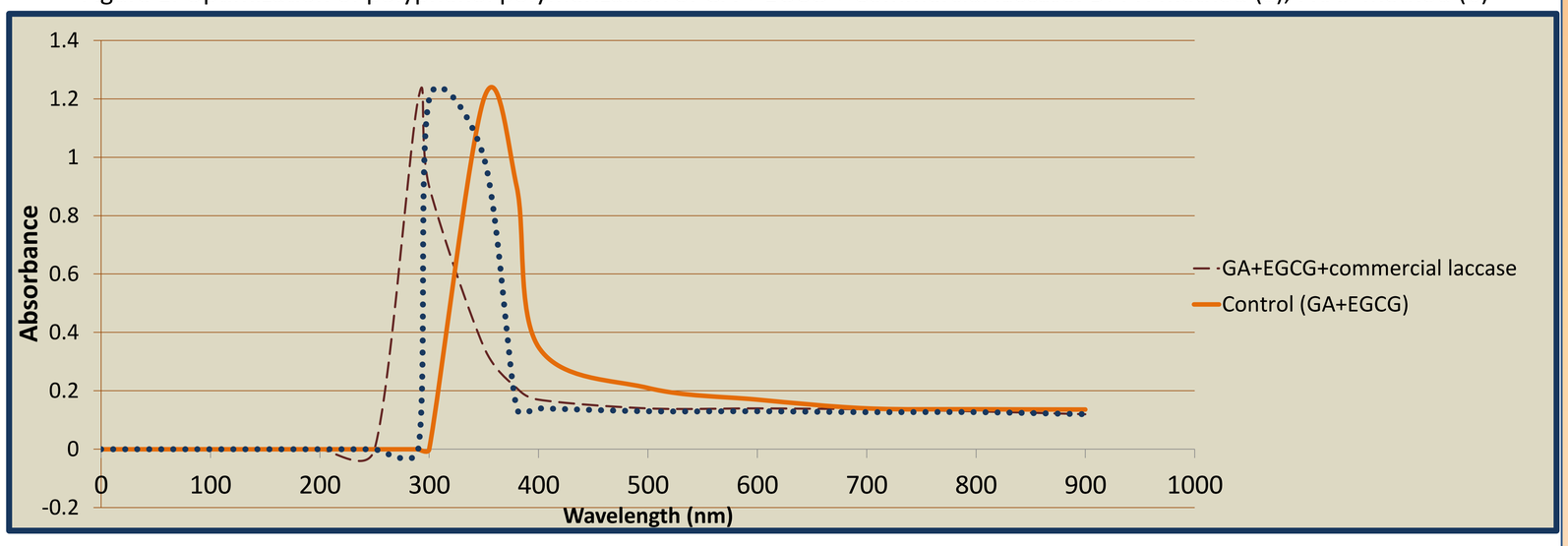


Figure 3. UV-Vis spectrum of three different samples containing laccases and mixture of polyphenols

The spectrophotometric analysis indicate that both of laccases, commercial and laccase from *Ganoderma spp*. transformed the mixture of gallic acid and epigallocatechin gallate (Fig. 3).

CONCLUSION

The mixture of GA and EGCG polyphenols and 0.3 U/ml of laccase expressed the highest antioxidant activity after 14 h of incubation, which indicates that detailed optimization is very important for obtaining of products with desirable high antioxidant activity.

Acknowledgements

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