

PEP ALHEIRA

Revestimentos edíveis ativos baseados nas proteínas do soro do leite e peptídeos antimicrobianos seus derivados para a indústria da alheira

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Resumo

Alheira é uma carne fumada da região Norte de Portugal (Trás-os-Montes) que foi nomeada como uma das 7 maravilhas da gastronomia portuguesa, sendo um verdadeiro “cartão de visita” de Trás-os-Montes e muito apreciado em Portugal e internacionalmente.

Devido à importância económica que a Alheira representa para a região de Trás-os-Montes, onde são produzidas anualmente mais de 500 toneladas, há um interesse crescente desta indústria em estender a vida útil da Alheira para evitar perdas económicas e expandir o produto para novos mercados internacionais, incluindo no exterior. Neste contexto, este projeto visa desenvolver um revestimento comestível ativo baseado em proteínas de soro de leite, um subproduto da indústria do queijo, e incorporar peptídeos derivados de proteínas do leite que funcionarão como agentes antimicrobianos.

Ao todo, este projeto apresenta um elevado potencial com benefícios financeiros diretos no desenvolvimento de um novo produto e na sua consequente valorização e competitividade em novos mercados.

Abstract

Alheira is a smoked meat from the North of Portugal (“Trás-os-Montes”) that has been named as one of the 7 wonders of Portuguese gastronomy, being a true “business card” of Trás-os-Montes and very appreciated in Portugal and internationally.

Due to the economic importance that Alheira represents for the region of Trás-os-Montes, where more than 500 tons are produced annually, there is a growing interest in this industry in extending the shelf-life of Alheira to avoid economic losses and expand the product to new international markets. In this context, this project aims to develop an active edible coating based on whey proteins, a by-product of the cheese industry, and to incorporate peptides derived from milk proteins that will act as antimicrobial agents.

Altogether, this project has a high potential with direct financial benefits in the development of a new product and its consequent appreciation and competitiveness in new markets.

Introduction

Food is normally susceptible to physical, chemical and microbiological deterioration throughout storage and distribution, (Cha & Chinnan, 2004) leading to a constant search for new strategies to increase food’s shelf-life. (Cha & Chinnan, 2004; Livney, 2015). Consequently, packaging became one of the most relevant areas in food industry (FI), where a great investment from the Science, Technology and Industrial sectors has been applied. (Cha & Chinnan, 2004; Livney, 2015). Furthermore, the current increase in consumer demand for natural ‘organic’ foods has forced companies and researchers to explore different ways to improve their market penetration by offering products with improvements in freshness, quality and food safety. (Peelman et al., 2013) One of the most fashionable trends consists of the development of innovative biopolymers based on natural polysaccharides, proteins or lipids obtained from by-products of the FI. (Valdes, Mellinas, Ramos, Garrigos, & Jimenez, 2014)

Therefore, the use of such biopolymers in food packaging applications has emerged as an alternative with regard to their film-forming properties to produce edible films and coatings (EFC) and as an environmentally friendly technology. (Cha & Chinnan, 2004; Livney, 2015; Peelman et al., 2013; Umaraw & Verma, 2017; Valdes et al., 2014) These biopolymers offer extra advantages such as barrier properties to gases and/or moisture, biocompatibility, nontoxicity, and low cost. (Silva-Weiss, Ihl, Sobral, Gomez-Guillen, & Bifani, 2013) Additionally, they can act as carriers for antimicrobial additives to extend food's shelf-life and safety of packaged foods, by reducing and/or preventing growth of pathogenic and spoilage microorganisms and, thus, leading to active edible films and coatings (AEFC). (Cagri, Ustunol, & Ryser, 2004) Noteworthy, the introduction of natural active additives to packaging materials provides advantages compared to the direct addition to food, such as the lower amount of active substances required, controlled release to food, and elimination of additional steps on processing. (Ramos, Jimenez, Peltzer, & Garrigos, 2014) whey proteins (WP) have been successfully employed as raw material for AEFC because they come from a renewable source and are a by-product of the cheese-manufacturing industry. Hence, they are widely available, relatively easy to handle and essentially inexpensive. Noteworthy, WP have shown promising mechanical features, as well as moisture and gas barrier properties comparable to those exhibited by the best synthetic polymer-based films. (Khwaldia, Perez, Banon, Desobry, & Hardy, 2004) Besides, WP-based films proved excellent biomaterials for use as carriers of food additives, as antimicrobials, improving the functionality of the packaging by bringing about novel features. (Ciesla, Salmieri, & Lacroix, 2006)

In current FI approaches to AEFC, additives are usually added as free components to the EFC mixture, such as natural preservatives (e.g. bacteriocins), or synthetic antibiotics (e.g. enilconazole). (Cagri et al., 2004) Recent trends point out antimicrobial peptides (AMP) as promising alternatives to current food preservatives. (Espitia et al., 2012) AMP are well-known components of the innate immune system that are rapidly gaining relevance, as opposed to conventional antibiotics whose effectiveness is declining. (Theolier, Fliss, Jean, & Hammami, 2014) This is explained by a group of special features, including wide activity spectrum, high efficacy at low concentrations, and low propensity for eliciting resistant microbial strains. (Costa, Carvalho, Montelaro, Gomes, & Martins, 2011). Strictly, AMP are not as new as food preservatives since bacteriocins, as pediocin, a 62-residue naturally occurring AMP, are of wide use in the FI. (Cagri et al., 2004) However, smaller AMP have not been yet explored as food preservatives, although they might represent cheaper alternatives to bacteriocins in use. Moreover, small peptides are usually non-immunogenic, so

they do not usually trigger allergic reactions as proteins or longer peptides may do. (Costa et al., 2011) In this context, AMP that might be of particular interest for the FI are the ones derived from bovine lactoferrin (bLf),(Theolier et al., 2014) a protein that occurs naturally in cow's milk, and whose use as nutraceutical has been recently considered as safe by the European Food Safety Authority. (Albano, Henriques, Correia, Hogg, & Teixeira, 2008).

The potential of bLf-derived AMP as key components in AEFC, through its addition to the coating solution, has never been reported and seems to be worth exploring. Alheira, 'the King of Portuguese sausage', is a typical smoked sausage of the Northern region in Portugal (Trás-os-Montes) that was nominated as one of the 7 wonders of Portuguese gastronomy, being a true 'calling card' of Trás-os-Montes and greatly appreciated in Portugal and internationally. (Albano et al., 2008) Alheira is produced from pork and poultry meat, and pork fat, wheat bread and olive oil. Ingredients are mixed with salt, garlic and spices until they form a paste, which is then stuffed into natural or artificial casings and submitted to a smoking process for no longer than 8 days. (Albano et al., 2008) Alheira shelf-life is about 1 month if stored at 4 °C in air or longer if the sausages are packed under modified atmosphere (ca. 60 days) or vacuum (ca. 90 days). Due to the economic importance that Alheira represents for Trás-os-Montes region, where more than 500 tons are produced annually, there is an increasing interest from this industry to extend Alheira shelf-life to avoid economic losses and to expand the product into new international markets, including overseas.

The development of AEFC on meat food products have been subject of a great number of scientific publications and patents during the last decade. (Korhonen & Pihlanto, 2003) However, there is a wide diversity of meat products with different characteristics making it difficult to standardize a single AEFC application procedure. Thus, there is the need to develop the appropriate AEFC for a specific meat product. Altogether, the above shows that increasing Alheira shelf-life by developing an active edible coating produced from WP, a by-product of the cheese-manufacturing industry, incorporating bLf-derived AMP as antimicrobial agents, is a truly innovative approach that may become a breakthrough for the FI.

References

- Albano, H., Henriques, I., Correia, A., Hogg, T., & Teixeira, P. (2008). Characterization of microbial population of 'Alheira' (a traditional Portuguese fermented sausage) by PCR-DGGE and traditional cultural microbiological methods. *Journal of Applied Microbiology*, 105(6), 2187-2194. doi:10.1111/j.1365-2672.2008.03947.x.
- Cagri, A., Ustunol, Z., & Ryser, E. T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection*, 67(4), 833-848. doi:10.4315/0362-028x-67.4.833.
- Cha, D. S., & Chinnan, M. S. (2004). Biopolymer-based antimicrobial packaging: A review. *Critical Reviews in Food Science and Nutrition*, 44(4), 223-237. doi:10.1080/10408690490464276.
- Ciesla, K., Salmieri, S., & Lacroix, M. (2006). Modification of the properties of milk protein films by gamma radiation and polysaccharide addition. *Journal of the Science of Food and Agriculture*, 86(6), 908-914. doi:10.1002/jsfa.2436.
- Costa, F., Carvalho, I. F., Montelaro, R. C., Gomes, P., & Martins, M. C. L. (2011). Covalent immobilization of antimicrobial peptides (AMPs) onto biomaterial surfaces. *Acta Biomaterialia*, 7(4), 1431-1440. doi:10.1016/j.actbio.2010.11.005.
- Espitia, P. J. P., Soares, N. D. F., Coimbra, J. S. D., de Andrade, N. J., Cruz, R. S., & Medeiros, E. A. A. (2012). Bioactive Peptides: Synthesis, Properties, and Applications in the Packaging and Preservation of Food. *Comprehensive Reviews in Food Science and Food Safety*, 11(2), 187-204. doi:10.1111/j.1541-4337.2011.00179.x.
- Khwalidia, K., Perez, C., Banon, S., Desobry, S., & Hardy, J. (2004). Milk proteins for edible films and coatings. *Critical Reviews in Food Science and Nutrition*, 44(4), 239-251. doi:10.1080/10408690490464906.
- Korhonen, H., & Pihlanto, A. (2003). Food-derived bioactive peptides - Opportunities for designing future foods. *Current Pharmaceutical Design*, 9(16), 1297-1308. doi:10.2174/1381612033454892.
- Livney, Y. D. (2015). Nanostructured delivery systems in food: latest developments and potential future directions. *Current Opinion in Food Science*, 3, 125-135. doi:10.1016/j.cofs.2015.06.010.
- Peelman, N., Ragaert, P., De Meulenaer, B., Adons, D., Peeters, R., Cardon, L., . . . Devlieghere, F. (2013). Application of bioplastics for food packaging. *Trends in*

Food Science & Technology, 32(2), 128-141. doi:10.1016/j.tifs.2013.06.003.

Ramos, M., Jimenez, A., Peltzer, M., & Garrigos, M. C. (2014). Development of novel nano-biocomposite antioxidant films based on poly (lactic acid) and thymol for active packaging. Food Chemistry, 162, 149-155. doi:10.1016/j.foodchem.2014.04.026.

Silva-Weiss, A., Ihl, M., Sobral, P. J. A., Gomez-Guillen, M. C., & Bifani, V. (2013). Natural Additives in Bioactive Edible Films and Coatings: Functionality and Applications in Foods. Food Engineering Reviews, 5(4), 200-216. doi:10.1007/s12393-013-9072-5.

Theolier, J., Fliss, I., Jean, J., & Hammami, R. (2014). MilkAMP: a comprehensive database of antimicrobial peptides of dairy origin. Dairy Science & Technology, 94(2), 181-193. doi:10.1007/s13594-013-0153-2.

Umaraw, P., & Verma, A. K. (2017). Comprehensive review on application of edible film on meat and meat products: An eco-friendly approach. Critical Reviews in Food Science and Nutrition, 57(6), 1270-1279. doi:10.1080/10408398.2014.986563.

Valdes, A., Mellinas, A. C., Ramos, M., Garrigos, M. C., & Jimenez, A. (2014). Natural additives and agricultural wastes in biopolymer formulations for food packaging. Frontiers in Chemistry, 2. doi:10.3389/fchem.2014.00006.