**ACTIVE EVOH/PE BAG FOR SLICED PAN LOAF BASED ON GARLIC AS
ANTIFUNGAL AGENT AND BREAD AROMA AS AROMA CORRECTOR.**

**Raquel Heras-Mozos, Virginia Muriel-Galet, Gracia López-Carballo, Ramón Catalá, Pilar
Hernández-Muñoz, Rafael Gavara***

**Packaging Group, Instituto de Agroquímica y Tecnología de Alimentos, IATA-CSIC, Av.
Agustin Escardino 7, 46980 Paterna (SPAIN)**

ABSTRACT

The aim of this work was the industrial development of an antifungal film containing garlic extract and bread aroma to be applied as active packaging for preservative-free sliced pan loaf. The material consisted of LDPE coated with ethylene vinyl alcohol copolymer. First the coating formulation was optimized to be suitable for flexographic equipment. A 1 µm coating of EVOH containing the agents was applied onto the LDPE film. Garlic extract release provides the antimicrobial capacity while bread aroma masks the inappropriate garlic aroma. The optimized formulation contained 8% of garlic extract and 8% of bread aroma. Bags were prepared and used to pack sliced pan loaves. Mould development was delayed from 6 to 12 days by use of the active bag. Sensory and textural characterization of the food also revealed that the bread maintained its characteristics, and sensory panellists considered the product acceptable and with good purchase intention.

Keywords: garlic extract; film coating; antimicrobial packaging; sliced pan loaf; sensory acceptability

27 **1. INTRODUCTION**

28 Bread is an essential food product worldwide made by baking a dough obtained by mixing flour
29 and water and fermented by strains of *Saccharomyces cerevisiae*. Bread slowly deteriorates after
30 baking, owing to bread staling and microbiological spoilage. Pan loaf is a type of bread with a
31 soft texture and high water activity ($0.94 < a_w < 0.97$) (Legan, 1993), commonly commercialized
32 sliced and bagged to delay staling and dehydration, but this also provides excellent conditions for
33 fungal growth and generation of mycotoxins (Filtenborg, Frisvad, & Thrane, 1996), since fungal
34 spores (such as *Penicillium* species) resist baking (Dagnas, Onno, & Membré, 2014). Sodium
35 propionate (E-281), sorbic acid (E-200), sodium sorbate (E-201), potassium sorbate (E-202),
36 calcium acetate (E-263) or ascorbic acid (E 300) (Guynot, Ramos, Sanchis, & Marín, 2005) are
37 added to dough to reduce fungal growth and increase bread shelf life. Owing to consumer demand
38 for preservative-free products, companies are looking for alternative ways to inhibit mould
39 growth, including the development of active packaging incorporating antimicrobial agents.

40 Antimicrobial packaging is a food technology in which interactions between some components
41 of the packaging system and the food or food-surrounding environment improve the food's safety
42 and quality and increase its shelf life by impeding or inhibiting microbial growth and retarding
43 food deterioration. Compared to direct addition of preservatives, the great advantage of
44 antimicrobial packaging is that the system gradually releases the active agent on the food surface
45 where it is most needed (Balaguer, Lopez-Carballo, Catala, Gavara, & Hernandez-Munoz, 2013).
46 At present, the preferred antimicrobial agents for packaging are natural components such as plants
47 extracts and essential oils (Ribeiro-Santos, Andrade, Melo, & Sanches-Silva, 2017). Oregano,
48 mustard, anise or cinnamon essential oils or garlic extract have been reported to show lethal or
49 inhibitory activity against various microorganisms in food (Giteru, Oey, Ali, Johnson, & Fang,
50 2017; Higuera, Lopez-Carballo, Hernandez-Munoz, Catala, & Gavara, 2014; Johnson, Olaleye,
51 & Kolawole, 2016; Ju, et al., 2018; Matan, Matan, & Ketsa, 2012; Teixeira, et al., 2014). Several
52 sulfur compounds are responsible for the antimicrobial activity in garlic (Borlinghaus, Albrecht,
53 Gruhlke, Nwachukwu, & Slusarenko, 2014; Kyung, 2012).

54 In a previous work (Heras-Mozos, Muriel-Galet, López-Carballo, Hernández-Muñoz, & Gavara,
55 2018), garlic extract was reported to be an excellent inhibitory agent against *Penicillium*
56 *expansum* in pan loaf. In that paper, LDPE film was coated with zein, EVOH and PE and used to
57 package bread slices. The results showed that zein coatings provided an excellent inhibitory
58 activity against mould growth. However, industrial production of that particular coating was
59 unfeasible because the final coating was very thick and rigid, modifying the mechanical properties
60 unacceptably, the film solution was too viscous to be applicable in an industrial printing or coating

technology and the odourless, colourless zein used is an expensive material for packaging purposes. Considering that polyethylene film coated with EVOH incorporating garlic extract also provided a considerable improvement of bread stability (Heras-Mozos, et al., 2018), EVOH was used in the present work to produce active packaging for sliced pan loaf. The known effect of the sensory impact caused by the addition of plant essential oils to products (garlic extract has a characteristic pungent aroma) was counteracted by bread crust aroma, used as an aroma-masking product.

This work reports the preparation and development of active packaging based on PE film coated with EVOH and incorporating garlic extract and bread crust aroma, its application in the manufacture of bags, and the resulting effect on additive-free sliced pan loaf in terms of microbial, textural and sensory attributes.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

A poly(ethylene-co-vinyl alcohol) with 29% ethylene molar content, Soarnol 2908 (EVOH), was kindly provided by The Nippon Synthetic Chemical Company (Osaka, Japan). Transparent 35 μ m-thick low density polyethylene (PE) was provided by Ducplast (Pobla del Duc, Spain). 1-Propanol was acquired from Sigma (Madrid, Spain). Garlic extract (GE) was kindly provided by DOMCA (Granada, Spain) and bread aroma (BA) containing 2-acetyl-1-pyrroline by Juan y Juan S.L. (Gandia, Spain). Water was obtained from a Milli-Q Plus purification system (Millipore, Molsheim, France).

2.2. Film preparation

Between 7 and 13 g of ethylene vinyl alcohol copolymer (EVOH) was dissolved in 100 mL of 1-propanol/water (50/50 w/w) at 75 °C under reflux and vigorous stirring. Once the polymer was fully dissolved, the solution was cooled to 40 °C and GE and BA were added to achieve the nominal concentration with respect to dry polymer (4 and 8% of each agent), and the mixture was stirred again for about 10 min. Control films were obtained without GE or BA.

Samples of LDPE film used to pack sliced wheat pan loaves were fixed to a glass plate. A corona treatment was applied with a BD-20AC high frequency corona surface treater (Electro-Technic Products, Inc., Chicago, IL, USA) for 2 minutes to improve the adhesion of the coating. The coating-forming solution was spread on the pretreated PE film using an extension bar with a 25- μ m-deep thread (Lin-Lab Rioja, Logroño, Spain) and immediately placed in a drying tunnel equipped with 3 250-W halogen lamps for 1 min. Finally, the films were stored in glass desiccators at 22 °C and in dry conditions (silica gel) prior to use. Film thickness was measured

with a C112-B Mitutoyo micrometer with a precision of 1 μm . Oxygen permeability at 90% RH was determined with an Oxtran 2/21 ML (Mocon, Minneapolis) with the procedure reported elsewhere (Cerisuelo, Gavara, & Hernandez-Munoz, 2014), using a 20 mm² test area.

Considering that the manufacturing procedure should be easily implemented in common industrial plastic-converting equipment, the viscosity of the final solution was optimized to be applied like inks in gravure or flexo printing machines. According to Ducplast (Pobla del Duc, Spain), where the coating trials were going to be carried out, the coating-forming solution should have a 25 s viscosity as measured with a No. 4 Ford cup. To complete the rheological characterization of the solution, a Rheostress 1 Thermo Haake rotational viscometer with a cone/plate system was used. The shear stress vs. shear rate data were collected and analysed with the equipment software.

For the industrial-scale preparation, a 25 L 7% EVOH solution was prepared in a large reactor. GE and BA were added at concentrations of 4 or 8% with respect to dry polymer weight. An LDPE film was coated on the corona-treated side with a 1- μm -thick uniform coating in an industrial flexographic printer (Ducplast, Spain). Standard sideweld bags made of LDPE or LDPE with the active coating were prepared by thermosealing with the same industrial conditions as used for standard LDPE film at Ducplast.

2.3. Bread production, packaging, storage and shelf life study

Preservative-free white pan loaves were produced, sliced and packaged at Juan y Juan (Gandia, Spain). The ingredients were white wheat, dehydrated yeast, water, salt and sugar. The dimensions of the 16 bread slices were approximately 12 cm $\times$ 12 cm and the total weight was around 500 g. Bags were made of standard LDPE film as it was or with the active EVOH coating. The antifungal effect was evaluated over time by visual inspection of fungal growth. Tests were performed in quintuplicate. Sensory and texture properties were also tested as described below.

2.4. Sensory analyses

To assess the acceptability of the product packaged in the active PE/EVOH bag containing garlic extract and bread crust aroma, two sensory tests were carried out in a standardized test room (UNE-ISO 8589, 2007). Slices were presented on plates covered with aluminium films and identified by three-digit codes.

First, a triangular test was carried out to determine whether the presence of garlic in the bread was noticeable. A total of 3 samples of bread in active and passive bags was presented to the panellists. The 42 untrained judges were asked to determine which of the three samples was different, and to indicate in the comment section what made it different. No information on the product was provided to the judges.

To assess the acceptability of the aroma, texture and taste of bread samples packaged in the active bag, a hedonic sensory test was carried out. Two slices were presented, one from a loaf packaged in the active bag and the other from a loaf in a conventional bag. The sensory test was carried out at day 7 since longer storage caused mould growth in bread packaged in the conventional bag.

The judges were asked to use a hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) to evaluate the sensory properties of the two bread samples, aroma, texture and taste (MacFie, Bratchell, Greenhoff, & Vallis, 1989). The panel consisted of 59 untrained members with the characteristics shown in Table 1. In this test, panel members were also asked for their purchase intention on a 1–5 scale (1 = I would never buy it, 5 = I would certainly buy it).

Table 1. Characteristics of the sensory panel with regard to sex, age and consumption habit.

Sex		Age		Consumption habit	
Female	39	Age < 30	32	Frequently	14
Male	20	31 < age < 45	16	Once a week	21
		46 < age	11	Once a month	14
				Rarely	10

2.5. Texture analysis

The texture analysis of the pan loaf packaged with the active and passive bags was conducted using a TA-XT plus Texture Analyser (Stable Micro Systems, Godalming, UK) with a 35 mm flat-end aluminium compression disc, coupled with a computer and the instrument software (Texture Expert v. 1.0). The samples were loaf slices tested in the crumb (10 slices per loaf). Following standard methods with some modifications (AACC, 2000; AIB, 1999), the samples were subjected to double compression at a speed of 1 mm/min. The compression process was conducted at constant deformation of the samples up to 40% of slice height, with a 5-second interval between consecutive compressions, and with a triggering force of 10 g.

The instrument software registered the force measured during the test and provided hardness (maximum force in N during the first compression), springiness (ratio between deformation at maximum compression during the second cycle and during the first cycle), which characterizes the degree of recovery, cohesiveness, which measures the internal bonding of the crumb (ratio of the areas (stress/strain) underneath the second and the first compression), and chewiness, which is the product of hardness, springiness and cohesiveness (Sanz, Salvador, Baixauli, & Fiszman, 2009).

2.6. Statistical analysis

Statistical analysis of the sensory data was performed by one-way ANOVA study. P-values of 0.05 or less were considered significant. The design of the sensory evaluation and the data analysis was carried out with Compusense Five software (release 5.0, Ontario, Canada).

3. RESULTS AND DISCUSSION

As described above, this work focuses on the development of an active bag for sliced wheat pan loaf, based on an LDPE film coated with EVOH incorporating garlic extract as antifungal agent and bread aroma to reduce any potential sensory effect on the bread. In a previous work (Heras-Mozos, et al., 2018), a PE bag with an 8 µm EVOH coating incorporating 0.5% of GE and 0.5% of BA was able to inhibit *P. expansum* growth on a single bread slice. In the present work, the development of an active packaging that could be manufactured industrially and be valid for active packaging of a whole sliced pan loaf was attempted. The presence of several sulfur compounds in the garlic extract was considered responsible for the antifungal activity of the extract (Casella, Leonardi, Melai, Fratini, & Pistelli, 2013; Heras-Mozos, et al., 2018). Bread aroma containing several compounds responsible for the baked aroma was used to mask the garlic flavour (Cho & Peterson, 2010).

3.1. Adjustment of EVOH coating

To adjust the rheology of the coating solution to the flexography parameters, a viscosity equivalent to 25 s in a No. 4 Ford cup, several EVOH solutions were prepared with EVOH concentrations ranging from 7 to 13%. Table 2 resumes the results obtained with the Ford cup, which indicate that the polymer concentration to obtain a varnish suitable for flexography would be 7%. In order to better understand the rheological behaviour of the product, the shear stress (σ) of the same solutions was analysed as a function of the shear rate ($\dot{\gamma}$). According to the Ostwald–de Waele law:

$$\sigma = K \cdot \dot{\gamma}^n \quad (1)$$

where K is the consistency index and n, the flow behaviour index, expresses the type of fluid analysed; and n=1 indicates a Newtonian fluid, n<1 a pseudoplastic fluid, and n>1 a dilatant fluid. The data obtained after fitting of the experimental results are also shown in Table 2. As can be seen, the EVOH solutions presented a Newtonian behaviour (n≈1).

Table 2. Effect of EVOH concentration on viscosity of polymer solutions, measured in a No. 4 Ford cup, and parameters of the Ostwald–de Waele equation.

	No. 4 Ford cup	Ostwald–de Waele parameters	
Concentration (w/w)	time (s)	k	n
7%	22.0 ± 0.5	0.083 ± 0.020	0.973 ± 0.004
8%	29.0 ± 0.5	0.077 ± 0.014	0.997 ± 0.004
9%	37.0 ± 0.5	0.126 ± 0.006	0.992 ± 0.009
11%	55.3 ± 0.6	0.225 ± 0.003	1.001 ± 0.001
13%	94.7 ± 1.2	0.405 ± 0.006	1.005 ± 0.000

On the basis of this result, the coating solution for the industrial trials was prepared with a 7% concentration of EVOH. Since the wet coating load on the film surface was about 15 g/m², the final film could be expected to present a dry coating of ca. 1 µm. Coating trials were carried out at laboratory scale, using a 25 µm extension bar and obtaining a coating with a thickness of 1.2 ± 0.5 µm, measured by subtracting the thickness of the LDPE substrate from the final thickness. Thickness was also measured gravimetrically, by weighing 1 dm² of film with and without coating. Assuming a density of 1.2 g/cm³, thickness was confirmed as 1.2 ± 0.2 µm. Optically, the coating was colourless and transparent, and the coated side was practically indistinguishable from the uncoated one.

The thermal sealability of the film was checked in a Packer P-400-C impulse sealer. The LDPE film was perfectly sealed at 7 (on a 1–9 scale) with a continuous weld that was not openable without great deformation of the film. The same result was observed for the coated LDPE film.

EVOH is a copolymer commonly used to provide an oxygen barrier, and its use as a coating on LDPE film could greatly modify the permeance of the final product. For this reason, the oxygen transmission through the film was measured at 0 and 90% RH, the latter condition close to that of a real loaf package, with the coated side in contact with the nitrogen chamber. The results were 3500 cc/(m².day.atm) for the LDPE film, and 1 cc/(m².day.atm) for the EVOH-coated LDPE film. These results were foreseeable, despite the thinness of the coating, because of the excellent barrier properties of dry EVOH. However, in wet conditions (90% RH), the measured permeance was 97 cc/(m².day.atm). EVOH sorbs large amounts of water when exposed to wet environments, and consequently the matrix becomes plasticized, reducing the interchain cohesive energy and facilitating molecular diffusion and thus increasing permeability. Nevertheless, wet EVOH is still a much better barrier material than LDPE and, as observed in this work, a very thin coating causes a sharp decrease in film permeance. This effect is not relevant for this work since the bread is packaged with air in the headspace, but it could be relevant in other projects considering modified atmosphere packaging.

3.2. Films preparation

LDPE with an 8 μm EVOH coating containing 0.5% of GE and 0.5% of BA was reported to present a considerable antifungal activity in laboratory tests with a package containing a single bread slice (Heras-Mozos, et al., 2018). Since (as already explained above) industrial flexographic coatings are ca. 1 μm thick, the first tests were carried out with a nominal concentration of 4.0% of GE and 4.0% of BA in order to obtain a similar amount of the antifungal agent. Film coatings were obtained in the laboratory by using a 25 μm extension bar. The final surface density of the dry coating was 1.2 g/dm^2 , equivalent to a thickness of 1 μm assuming a density of 1.2 g/cm^3 (EVOH general information). Information on the actual concentration of the agents after drying of the coating was attempted by thermal desorption and GC. With methods based on desorption and gas chromatographic analysis of EVOH coatings with other volatile agents (carvacrol, citral) it was reported that losses of about 50% of the nominal concentration are possible (Cerisuelo, et al., 2014). In this work, since the selected agents are very complex mixtures, thermal desorption coupled with GC did not provide relevant information. Instead, three samples of 4 dm^2 of coated PE incorporating GE (no BA was added) were stored in an oven for 1 week at 90 $^{\circ}\text{C}$ and weighed daily until constant weight was obtained. An identical sample without GE was tested in parallel. The results show that the EVOH coating retained $8 \pm 1\%$ of solvent after drying, and that the final GE in the coating was $2.6 \pm 1.0\%$.

3.3. Bread packaging, storage and shelf life study

The films, coated or uncoated, were employed to pack preservative-free sliced pan loaves. Sideweld bags were prepared with an industrial impulse thermosealer, using the conditions for LDPE film without modifications. The coating did not affect the sealing properties of the films.

Bread was baked, cooled to room temperature, sliced and packaged using atmospheric air as headspace, and closed with a twistband. Packages were stored at room temperature, and samples were visually inspected at days 6, 9, 12 and 15 to determine the antifungal effect of the package. The results are shown in Table 3. At day 6, all samples were free of moulds. Bread samples were tested by members of the laboratory to look for potential effects and to prepare the sensory test. In this informal test no relevant effect on bread aroma was observed, but some colleagues noticed some changes in texture.

At day 9, all loaves packaged in passive LDPE bags presented infection in at least one spot on the crust. Loaves packaged in the active LDPE/EVOH bags were free of mould growth. At day 12, the crust of loaves packaged in passive LDPE presented moulds and several slices presented mould growth in the crumb. Loaves in the active bags presented mould growth in at least one spot on the crust. At day 15, all loaves were spoiled by moulds, those in the passive bags with extensive invasion.

Table 3. Presence of moulds in preservative-free sliced pan loaf packaged in passive bags (LDPE) or active bags (LDPE/EVOH with 4% of garlic extract): (-), no growth; (+), growth in a few spots only affecting crust; (++) growth affecting crust and crumb: (+++) growth affecting large areas.

	Day 6	Day 9	Day 12	Day 15
Passive bag	-	+	++	+++
Active bag	-	-	+	++

From this first analysis it can be concluded that the release of garlic extract from the active package produced an antifungal effect against *P. expansum* in sliced pan loaf, although the improvement in stability from a microbiological point of view was only three days. To improve the efficiency of the package a new coating formulation was prepared, incorporating 8% of GE and 8% of BA. LDPE film was coated by flexography at Ducplast as indicated previously and with the same parameters. Bags were prepared and used to pack sliced loaves.

The active film obtained was highly transparent and thermosealable, like the one described previously. The effect of the active film on the microbiological stability of the bread was analysed visually and the results are presented in Table 4. As can be seen, the loaves in the passive bag presented stability up to 6 days, while bread in the active bag presented mould spoilage at day 15; thus, stability was extended to day 12. Figure 1 shows representative images of the loaves packaged in the passive and active bags.

Table 4. Presence of moulds in preservative-free sliced pan loaf packaged in passive bags (LDPE) or active bags (LDPE/EVOH with 8% of garlic extract): (-), no growth; (+), growth in a few spots only affecting crust; (++) growth affecting crust and crumb: (+++) growth affecting large areas.

	Day 6	Day 9	Day 12	Day 15
Passive bag	-	+	++	+++
Active bag	-	-	-	+

In a previous study (Heras-Mozos, et al., 2018), an active EVOH coating presented antifungal activity during at least 20 days, inhibiting mould growth, but tests were of a single slice in a bag, where the coating surface/bread mass ratio was very high. In the present study, the bag contains 18 slices and the total amount of active agent per bread mass is much lower. The interaction between the aroma and the bread, the potential sorption and the lower vapour pressure concentration in the headspace are factors that reduce the activity of the package (Gutiérrez, Batlle, Andújar, Sánchez, & Nerín, 2011).

Figure 1. Pictures of the evolution of the bread in the passive and active bags during storage.

3.4. Bread texture

In previous sensory tests carried out by members of the laboratory, some changes in texture were observed. The addition of the EVOH coating, a highly hydrophilic material, could lead to adsorption of water, promoting some bread drying. Therefore, bread samples were submitted to a texture analysis. Bread slices packaged in the standard and active bags were tested at days 5, 10 and 12. In the case of bread in the conventional package, some slices presented mould growth at the 10th and 12th days; therefore, clean slices were selected, that is, slices that were not infected and whose immediate neighbours were also not infected. The results for hardness, elasticity, cohesiveness and chewiness are shown in Figure 2. As can be seen, the hardness values for the bread packaged in active bags are slightly higher than those for bread in passive bags, although the differences are not significant. Indeed, none of the four parameters analysed experienced significant differences caused by the type of packaging.

Figure 2. Evolution of texture parameters (hardness, springiness, cohesiveness and chewiness) in the crumb of pan loaf slices of bread packaged in passive LDPE bags and in active LDPE/EVOH containing 8% of GE and BA.

3.5. Sensory analyses

Active packaging including oregano or cinnamon oils has been reported to inhibit mould growth in bread, but also to result in some detrimental effects on organoleptic properties owing to

inappropriate aroma (Balaguer, et al., 2013; Passarinho, et al., 2014). For volatile agents to be efficient as natural antimicrobials they must be at high concentrations (high activity), and therefore the effect on the aroma of the packaged product might be very considerable.

To discover whether this strategy was valid, a triangular test was carried out to determine whether consumers were able to distinguish between samples. Bread samples were baked, sliced and packaged in an industrial process (Juan y Juan S.L., Gandia, Spain) using a conventional bag or the active bag with 8% of GE and 8% of BA. The triangular analysis was carried out on day 3 to determine whether the garlic extract was exerting a significant impact on the sensory characteristics of the product. Judges were asked to smell three samples, two the same and one different, and identify the different one, if possible indicating what the difference was. No information was given to the judges about the differences between samples. According to standard analysis of triangular tests, for a panel of 42 judges samples could be considered significantly different (95% of confidence) with at least 20 correct answers. The results, shown in Table 5, indicated that the samples were indistinguishable since nearly two thirds of the judges indicated a wrong sample. The addition of the bread crust aroma masked the strong aroma of garlic, as Heras-Mozos, et al. (2018) reported elsewhere.

Table 5. Results of the triangular test to determine if consumers were able to distinguish bread packaged in active bags from bread packaged in passive bags.

Number of judges	Number of successes	Number of failures
42	16	26

A second sensory taste was carried out to determine acceptability and purchase intention. New pan loaves were prepared and packaged at Juan y Juan S.L. Samples were analysed at day 7 of storage. Longer periods of storage could not be tested because mould growth was present in bread packaged in conventional bags at day 9. The results of this test are shown in Table 6. As can be seen, the judges rated the product positively, with values above 5 in aroma, texture, taste and general acceptability. No significant differences were found in any of the tested characteristics between active and passive bags. With respect to purchase intention (1 to 5 scale), the test showed that the judges would possibly buy preservative-free pan loaf in active packages with GE, with the same intention as in the case of the conventional package, without significant differences. No comment about garlic perception was made by any of the panellists, which proves that the addition of the bread crust aroma provided the required masking effect.

Table 6. Results of the hedonic test of the acceptability of pan loaf slices (aroma, texture, taste and general acceptability) packaged in a conventional bag and in the active bag, and of the purchase intention for the product.

Sample	Acceptability				Purchase Intention (1-5)
	Aroma (1-9)	Texture (1-9)	Taste (1-9)	General (1-9)	
Conventional	6.54 ^a	6.51 ^a	6.34 ^a	6.51 ^a	3.47 ^a
Active	6.59 ^a	6.46 ^a	6.49 ^a	6.49 ^a	3.47 ^a

Therefore, the application of an active coating based on EVOH and containing an appropriate active agent is a suitable procedure for improving the stability of food products. In this work we have shown that its industrial implementation is feasible with standard printing/coating equipment. The considerable aromatic effect of some natural antimicrobial agents could be minimized by incorporating a masking agent: in this study, bread crust aroma. After optimization, a panel of judges considered the final product as acceptable for consumption and with good purchase intention. The final active package improves the product's stability from 6 to 12 days. Further improvement might be obtained by increasing the quantity of garlic extract incorporated, although a compromise between sensory and microbiological properties should be considered.

4. ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Spanish Ministry of Economy and Innovation, project AGL2015-64595-R, and Juan y Juan S.L., and R. H.-M.'s fellowship and the assistance of Mr. Karel Clapshaw (translation services).

5. REFERENCES

- AACC. (2000). Cereal laboratory methods. Bread firmness by universal testing machine. . American Association of Cereal Chemists, Method 74-09. In.
- AIB. (1999). AIB standard procedure. White pan bread. Kansas: American Inst. of Baking, Research Dept. American Inst. of Baking. . Method 02-31. In.
- Balaguer, M. P., Lopez-Carballo, G., Catala, R., Gava, R., & Hernandez-Munoz, P. (2013). Antifungal properties of gliadin films incorporating cinnamaldehyde and application in active food packaging of bread and cheese spread foodstuffs. *International Journal of Food Microbiology*, 166(3), 369-377.
- Borlinghaus, J., Albrecht, F., Gruhlke, M., Nwachukwu, I., & Slusarenko, A. (2014). Allicin: Chemistry and Biological Properties. *Molecules*, 19(8), 12591.
- Casella, S., Leonardi, M., Melai, B., Fratini, F., & Pistelli, L. (2013). The Role of Diallyl Sulfides and Dipropyl Sulfides in the In Vitro Antimicrobial Activity of the Essential Oil of Garlic, *Allium sativum* L., and Leek, *Allium porrum* L. *Phytotherapy Research*, 27(3), 380-383.
- Cerisuelo, J. P., Gava, R., & Hernandez-Munoz, P. (2014). Natural antimicrobial - containing EVOH coatings on PP and PET Films: functional and active property characterization. *Packaging Technology and Science*, 27(11), 901-920.

- Cho, I. H., & Peterson, D. G. (2010). Chemistry of bread aroma: A review. *Food Science and Biotechnology*, 19(3), 575-582.
- Dagnas, S., Onno, B., & Membré, J.-M. (2014). Modeling growth of three bakery product spoilage molds as a function of water activity, temperature and pH. *International Journal of Food Microbiology*, 186, 95-104.
- Filténborg, O., Frisvad, J. C., & Thrane, U. (1996). Moulds in food spoilage. *International Journal of Food Microbiology*, 33(1), 85-102.
- Giteru, S. G., Oey, I., Ali, M. A., Johnson, S. K., & Fang, Z. (2017). Effect of kafirin-based films incorporating citral and quercetin on storage of fresh chicken fillets. *Food Control*, 80, 37-44.
- Gutiérrez, L., Batlle, R., Andújar, S., Sánchez, C., & Nerín, C. (2011). Evaluation of Antimicrobial Active Packaging to Increase Shelf Life of Gluten-Free Sliced Bread. *Packaging Technology and Science*, 24(8), 485-494.
- Guynot, M. E., Ramos, A. J., Sanchis, V., & Marín, S. (2005). Study of benzoate, propionate, and sorbate salts as mould spoilage inhibitors on intermediate moisture bakery products of low pH (4.5–5.5). *International Journal of Food Microbiology*, 101(2), 161-168.
- Heras-Mozos, R., Muriel-Galet, V., López-Carballo, G., Hernández-Muñoz, P., & Gavara, R. (2018). Development and optimization of an antifungal packaging for sliced pan loaf based on garlic as agent and bread aroma as aroma corrector. *International Journal of Food Microbiology*, in press.
- Higueras, L., Lopez-Carballo, G., Hernandez-Munoz, P., Catala, R., & Gavara, R. (2014). Antimicrobial packaging of chicken fillets based on the release of carvacrol from chitosan/cyclodextrin films. *International Journal of Food Microbiology*, 188, 53-59.
- Johnson, M., Olaleye, O. N., & Kolawole, O. S. (2016). Antimicrobial and Antioxidant Properties of Aqueous Garlic (*Allium sativum*) Extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *British Microbiology Research Journal*, 14(1), 1-11.
- Ju, J., Xu, X., Xie, Y., Guo, Y., Cheng, Y., Qian, H., & Yao, W. (2018). Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. *Food Chemistry*, 240, 850-855.
- Kyung, K. H. (2012). Antimicrobial properties of allium species. *Current Opinion in Biotechnology*, 23(2), 142-147.
- Legan, J. D. (1993). Mould spoilage of bread: the problem and some solutions. *International Biodeterioration & Biodegradation*, 32(1), 33-53.
- MacFie, H. J., Bratchell, N., Greenhoff, K., & Vallis, L. V. (1989). Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *Journal of Sensory Studies*, 4(2), 129-148.
- Matan, N., Matan, N., & Ketsa, S. (2012). Effect of heat curing on antifungal activities of anise oil and garlic oil against *Aspergillus niger* on rubberwood. *International Biodeterioration & Biodegradation*, 75, 150-157.
- Passarinho, A. T. P., Dias, N. F., Camilloto, G. P., Cruz, R. S., Otoni, C. G., Moraes, A. R. F., & Soares, N. d. F. F. (2014). Sliced Bread Preservation through Oregano Essential Oil-Containing Sachet. *Journal of Food Process Engineering*, 37(1), 53-62.
- Ribeiro-Santos, R., Andrade, M., Melo, N. R. d., & Sanches-Silva, A. (2017). Use of essential oils in active food packaging: Recent advances and future trends. *Trends in Food Science & Technology*, 61, 132-140.
- Sanz, T., Salvador, A., Baixauli, R., & Fiszman, S. M. (2009). Evaluation of four types of resistant starch in muffins. II. Effects in texture, colour and consumer response. *European Food Research and Technology*, 229(2), 197-204.
- Teixeira, B., Marques, A., Pires, C., Ramos, C., Batista, I., Saraiva, J. A., & Nunes, M. L. (2014). Characterization of fish protein films incorporated with essential oils of clove, garlic and origanum: Physical, antioxidant and antibacterial properties. *Lwt-Food Science and Technology*, 59(1), 533-539.

429

430