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Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil

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Abstract

The effect of modified atmosphere packaging (MAP) on the most important spoilage fungi of bread was investigated. *Penicillium commune*, *P. roqueforti*, *Aspergillus flavus* and *Endomyces fibuliger* were able to grow at oxygen levels down to 0.03%, while the chalk mould *E. fibuliger* was capable of growing even in the presence of an oxygen absorber. High levels of carbon dioxide retarded growth but not completely. As an alternative to MAP active packaging (AP) using volatile essential oils (EO) and oleoresins (OL) from spices and herbs were tested against a range of fungi commonly found on bread. Concentrations of 1, 10 or 100 μl EO or OL were added to a filter paper placed in the lid of a Petri dish inoculated with one of the test fungi. The Petri dish was sealed hermetically to avoid the exchange of gases. Mustard essential oil showed the strongest effect. Cinnamon, garlic and clove also had high activity, while oregano oleoresin only inhibited growth weakly. Vanilla showed no inhibitory effect towards the tested microorganisms at the applied concentrations. *A. flavus* was more resistant than the other microorganisms while *P. roqueforti* was the most sensitive. Mustard essential oil was investigated in greater detail. The minimal inhibitory concentration (MIC) for the active component, allyl isothiocyanate (AITC), was determined for the same species and an additional three moulds and one yeast. MIC values ranged from 1.8 to 3.5 μg/ml gas phase. Results showed that whether AITC was fungistatic or fungicidal depended on its concentration, and the concentration of spores. When the gas phase contained at least 3.5 μg/ml, AITC was fungicidal to all tested fungi. Results of sensory evaluation showed, that hot-dog bread was more sensitive to AITC than rye bread. The minimal recognisable concentration of AITC was 2.4 μg/ml gas phase for rye bread and between 1.8 and 3.5 μg/ml gas phase for hot-dog bread. These findings showed that the required shelf-life of rye bread could be achieved by active packaging with AITC. Active packaging of hot-dog bread, may nevertheless require the additional effect of other preserving factors to avoid off-flavour formation

Keywords: Active packaging; Natural antimicrobials; Spices; Bread; Fungi; Allyl isothiocyanate

1. Introduction

In bakery products, fungi are the most common spoilers. In unpreserved bread a shelf-life of 3–4 days may be expected especially if the hygiene in the

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factory is not sufficiently high. Besides the repelling sight of visible growth, fungi are responsible for off-flavour formation and the production of mycotoxins and allergenic compounds. These compounds may be formed even before growth is visible

In wheat bread *Penicillium commune, P. solitum, P. corylophilum* and *Aspergilllus flavus* dominates, whereas *P. roqueforti, P. corylophilum* and to some degree also *Eurotium* species are dominant in rye bread (Nielsen, unpublished results; Lund et al., 1996). Also, the yeast commonly known as chalk moulds are important spoilers of bread. The most important of these species is *Endomyces fibuliger*.

Since industrialisation, urbanisation and change of life style started to put demands for longer shelf-life of bread, the use of sorbate and propionic acid has been the main choice. However, today consumers demand less use of synthetic preservatives but still they expect food to be free from microbial growth, toxins and other quality deteriorating factors. Meanwhile product freshness and sensorial qualities must be preserved. The problem for the food industry is to fulfill the demands of minimum changes in food quality and maximum security.

Modified atmosphere packaging (MAP) may provide the additional shelf-life required, but results from our laboratory have shown that in several cases, especially when chalk moulds are involved, sufficient protection cannot be achieved. Best performance is obtained by the deep draw technique, which involves an evacuation of the package before it is flushed with the desired gas, however this has a much lower throughput than flow pack. Whereas flow pack results in a much higher oxygen level in the final pack, as it is just forcing out the air surrounding the product by blowing the package gas into the package just before it is sealed off. The spongy structure of bread thus results in residual oxygen content of 3–5%, which is insufficient to retard fungal growth (Haasum and Nielsen, 1998).

Active packaging may be an interesting alternative to both the traditional use of preservative and MAP. It involves incorporation of agents in the package. These agents can either interact directly with the spoilage organisms or interact with the environment inside the package. Several types of active packaging has been described. Here the use of oxygen absorbers are most known and is gaining popularity, whereas the use of natural antimicrobials is still in its infancy (Floros et al., 1997).

Several spices, herbs and fruits contains volatile anti-microbial compounds (Zaika, 1988). These may find use in active packaging. Several studies revealing results on the preservative action of spices or their essential oils have been made (Azzouz and Bullerman, 1982; Shelef, 1983; Conner and Beuchat, 1984; Zaika, 1988). Although different results are observed depending on test conditions, microorganisms and source of the antimicrobial compound, some spices or essential oils always act very effectively in inhibiting growth. One of these is mustard essential oil, which primarily contains the active compound allyl isothiocyanate (AITC). Allyl isothiocyanate exists in a precursor form, which by cell destruction is enzymatically hydrolysed to release the active form. The precursor of allyl isothiocyanate is found in common plants such as mustard, broccoli, horseradish, cabbage, cauliflower, kale and turnips.

The objectives of this work were to study the inhibitory effect of selected essential oils and oleoresins against mould and yeast associated with bread. The extracts that were used are from cinnamon, garlic, clove, mustard, oregano and vanilla. Special interest was put on mustard essential oil (90–95% pure AITC), in order to determine the minimum inhibitory concentration, and evaluate the potential of using AITC in active packaging of bread. This also involved the sensory evaluation of bread packed with AITC.

2. Materials and methods

2.1. Test microorganisms

Microorganisms used for the experiment were obtained from the IBT culture collection at the Department of Biotechnology, Technical University of Denmark. All the fungi and yeast were isolated from bread and are listed in Table 1.

2.2. Media

All growth experiments were carried out on the standard medium for fungi, Czapek yeast extract

Table 1
Microorganisms used in the experiment

Taxa	Culture collection no.	Source	Test
Aspergillus flavus	IBT 15606	–	a
Aspergillus flavus	IBT 21315	Burger bun	b and c
Endomyces fibuliger	IBT 605	Rye bread	a
Endomyces fibuliger	IBT 631	Rye bread	b and c
Penicillium commune	IBT 10253	Cheese	a
Penicillium commune	IBT 21314	Hot-dog bread	b and c
Penicillium corylophilum	IBT 21310	Hot-dog bread	a and c
Penicillium discolor	IBT 13777	Cheese	c
Penicillium palitans	IBT 21309	Hot-dog bread	c
Penicillium polonicum	IBT 21317	Hot-dog bread	c
Penicillium roqueforti	IBT 12845	Cheese	a
Penicillium roqueforti	IBT 21322	Hot-dog bread	b and c
Penicillium solitum	IBT 21313	Hot-dog bread	b and c
Pichia anomala	IBT 603	Rye bread	c

[a–c] (a) Modified atmosphere study, (b) screening essential oils and oleoresins, (c) sensory evaluation of active packaged bread.

agar (CYA), with the addition of trace metals (Samson et al., 1995).

2.3. Modified atmosphere study

The test organisms listed in Table 1 (test a) was inoculated on CYA plates and packed in a high barrier plastic bag (Ecotop 20/75 Transobar, a laminate of OPP20/EVOH/PELD45/PELLD30, Åkerlund and Rausing, Sweden) using a vacuum chamber packaging machine (Multivac A 300/42 MC, Germany). The following gases were used: pure nitrogen and carbon dioxide containing less than 0.002% residual oxygen and pure nitrogen or carbon dioxide to which 0.5 or 1.0% oxygen had been added (AGA Gas AB, Sundbyberg, Sweden). The gases were either used directly or mixed 50:50% using a gas mixer (MAP MIX 9000, PBI-Dansensor A/S, Ringsted, Denmark). The mixing was controlled by a gas analyser (CheckMate 900 O2/CO2, PBI-Dansensor A/S, Ringsted, Denmark).

2.4. Essential oils and oleoresins

The essential oils and oleoresins used in this study are listed in Table 2.

2.5. Preparations of suspensions of test microorganisms

Fungal conidia and yeast vegetative cells were harvested after inoculation on CYA for 7 days at 25°C, by an inoculation needle with a loop (3 mm) and transferred to a test-tube with a spore suspension media (0.5% Tween 80, 0.5% agar in distilled water)

Table 2
Essential oils and oleoresins used in the experiment.

Compound	Product no.	Manufacturer	Purity
Mustard essential oil	ETS20100	Ruther & Saecker, Germany	90–95%
Cinnamon oleoresin	Not specified	Bush Boake Allen, UK	Not specified
Oregano oleoresin	OLO10225	Ruther & Saecker, Germany	Not specified
Clove oleoresin	225458	Bush Boake Allen, UK	70–83% v/w
Vanilla oleoresin	OLV10044	Ruther & Saecker, Germany	1.3–1.5%
Garlic essential oil	ETK25098	Ruther & Saecker, Germany	Not specified

2.6. Screening essential oils and oleoresins

Screening of the essential oils and oleoresins were done by three-point inoculations of the microorganisms on CYA in a standard Petri dish from a spore suspension as described above. A sterilised filter paper disk (Whatman type 1, 2 cm in diameter, Struers Kebo Lab, Denmark) were placed centred on the inside of a Petri dish cover and 1, 10 or 100 μl of one of each essential oil or oleoresin were added to the filter. Controls were made by adding 100 μl water to a filter paper. All experiments were performed in duplicate. The plates were incubated in reversed position at 25°C in the dark. Colony diameter was measured after 3, 5, 7 and 14 days. The microorganisms used are listed in Table 1 (test b).

2.7. Minimal inhibitory concentration of AITC

Three-point inoculations of the microorganisms were made from a spore suspension as described above, with 60 ml CYA in 250-ml culture flasks. With the agar side up, a sterilised filter paper disk (Whatman type 1, 2 cm in diameter, Struers Kebo Lab, Denmark) were placed in the bottom centred on the inside of the culture flask. AITC was serial diluted in ethanol and 10 μl of each dilution were added to the filter. The plates were closed with a screw lid, resulting in different concentrations of AITC in the headspace. In the control flasks, 10 μl ethanol was added to the filter paper. An extra control without the screw lid (open control) was prepared to test the effect of closing the system. All experiments were performed in duplicate. The plates/culture flasks were incubated in reversed position at 25°C in the dark. Colony diameter was measured after 3, 5, 7 and 14 days. At day 14, the screw lid were unscrewed and incubation were continued. Colony diameters were measured after 17, 19, 21 and 28 days from the day of inoculation. The microorganisms used are listed in Table 1 (test c).

2.8. Sensory evaluation of active packaged bread

One piece of rye bread (Møllens Brød A/S, Helsingør, Denmark) or one slice of hot-dog bread (Schulstad Brød A/S, Hvidovre, Denmark) was placed in a high barrier bag (PA/EVOH/PA, Product nr.20-563, Danisco flexible, Lyngby, Denmark) without having any contact with the bread. Three different volumes of AITC (0.5, 1.0 and 2.0 μl) and an empty control were added to the bags. The bags were then sealed hermetically. The packaging and sealing procedure were standardised so the volumes of the packages were reproducible. The packed breads were stored for 24 h at room temperature before the sensorial evaluation. Sensorial tests were made with an untrained panel, who tested both rye bread and hot-dog bread by ranking test. The descriptors used were: acidic flavour, sweet flavour, pungent taste and aroma. Sensorial evaluations were done in rooms specially designed for the purpose.

2.9. Data analysis

The relative growth were expressed as $(D_{EO/OL}/D_{control}) \cdot 100\%$, were $D_{EO/OL}$ is the mean diameter measured for a fungus grown with an essential oil (EO) or oleoresin (OL) at a given concentration and day. $D_{control}$ is the diameter measured for the control fungus at the same conditions.

Sensorial data were analysed by analysis of variance. Soerensen et al. (1978) describes a method that utilises data from a ranking test in a variance analysis. For multivariable data analysis sensorial data were analysed using principal component analysis (PCA). which is good for situations with many correlated input variables and several result variables (Haasum and Nielsen, 1998). The *X* matrix consists of the number, which represents the judges, and the concentrations of AITC used. The *Y* matrix contains the response variables, that are the marks given for the taste and aroma of rye- and hot-dog bread for the respective flavours, given by the judges. Results are showed in a loading plot which explains the relationship between each variable and how significant each variable is for each principal component (PC). Significance of the PCs was tested by cross validation. The program SIMCA-P for Windows (version 3.01, Umetri AB & Ericsson Erisoft AB, Sweden) was used for the multivariate data analysis.

3. Results and discussion

3.1. Modified atmosphere study

Growth of the most important bread spoilage fungi

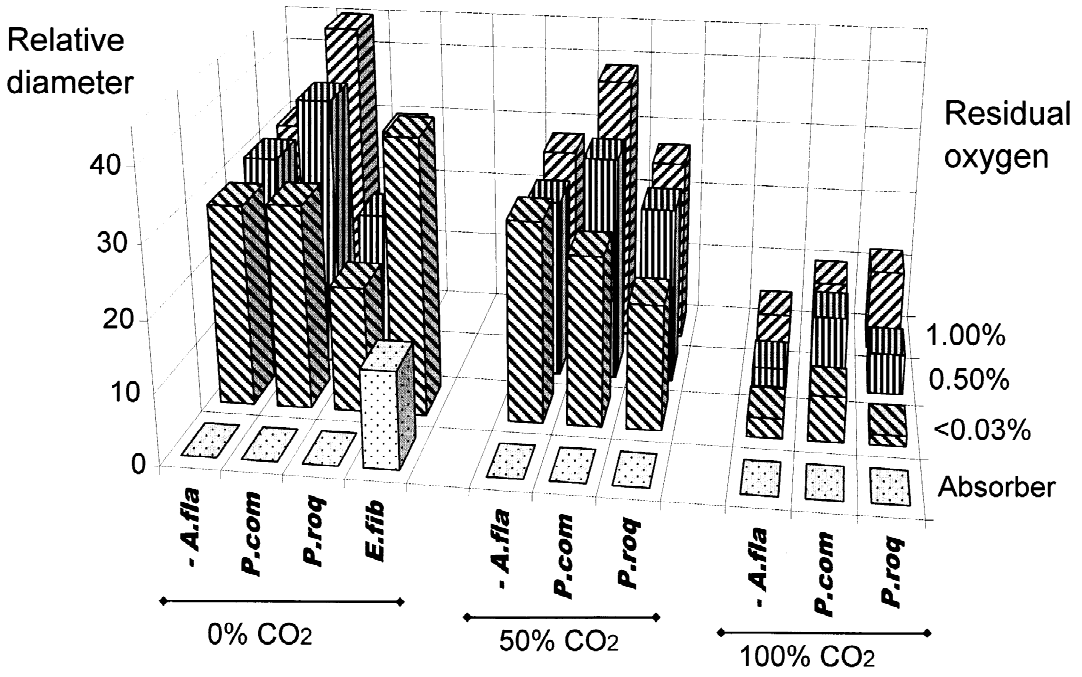

Fig. 1. Growth of the most important bread spoilage fungi in modified atmosphere packaging relative to growth in air (*Aspergillus flavus* (*A.fla*), *Penicillium commune* (*P.com*), *P. roqueforti* (*P.roq*) and *Endomyces fibuliger* (*E.fib*)). Three levels of residual oxygen (1.00, 0.50 and <0.03%) and a package with an oxygen absorber were used at three gas mixtures of nitrogen and carbon dioxide (100:0; 50:50 and 0:100).

when packaged in modified atmosphere is plotted in Fig. 1. This shows the growth at low residual oxygen levels relative to packaging in air. The yeast *Endomyces fibuliger* reached an average of 1.5 mm when cultured in a package with an oxygen absorber, and 4 mm at 0.02 and 0.1%. Relative to the control, which reached 11 mm in one week, the diameter was 14, 36 and 36%, respectively. The effect of increased carbon dioxide was not tested for this fungus. The moulds did not grow in the packages with oxygen absorbers but at 0.02–0.03% residual oxygen in either pure nitrogen or 50% nitrogen and 50% carbon dioxide their colony diameter reached 18–29% of the control. In pure carbon dioxide, the moulds were strongly inhibited reaching only 1–6% at 0.02–0.03% oxygen and 4–11% at 1.0% residual oxygen.

Growth of the fungi studied was clearly retarded at reduced oxygen levels, but only when an oxygen absorber was applied could mould growth be inhibited completely. Growth of the chalk mould yeast *E. fibuliger* was not completely inhibited by the anaerobic conditions obtained when an oxygen absorber was used. Thus growth of fungal contaminants may be anticipated on products package in modified atmosphere unless other preserving factors are used in combination with it. This was the reason for investigating possibility of utilising natural antimicrobials in packaging.

3.2. Screening essential oils and oleoresins

In Fig. 2, the antimicrobial effect of 1 μl of each essential oil and oleoresin after 7 days are plotted. Mustard essential oil (AITC) was the only compound strong enough to completely inhibit growth of all of the microorganisms for 7 days. Cinnamon, garlic and clove were all potential inhibitors, oregano oleoresin was only a weak inhibitor and vanilla did not inhibit the microorganisms at all at this concentration. *Aspergillus flavus* appeared to be the most resistant of the microorganisms. Besides mustard EO only clove OL decreased growth of *A. flavus* markedly (40% reduction). The rest of the antimicrobials had less than 10% inhibitory effect on *A. flavus*. In contrast *Penicillium roqueforti* were the most sensitive of the microorganisms tested. Only vanilla OL

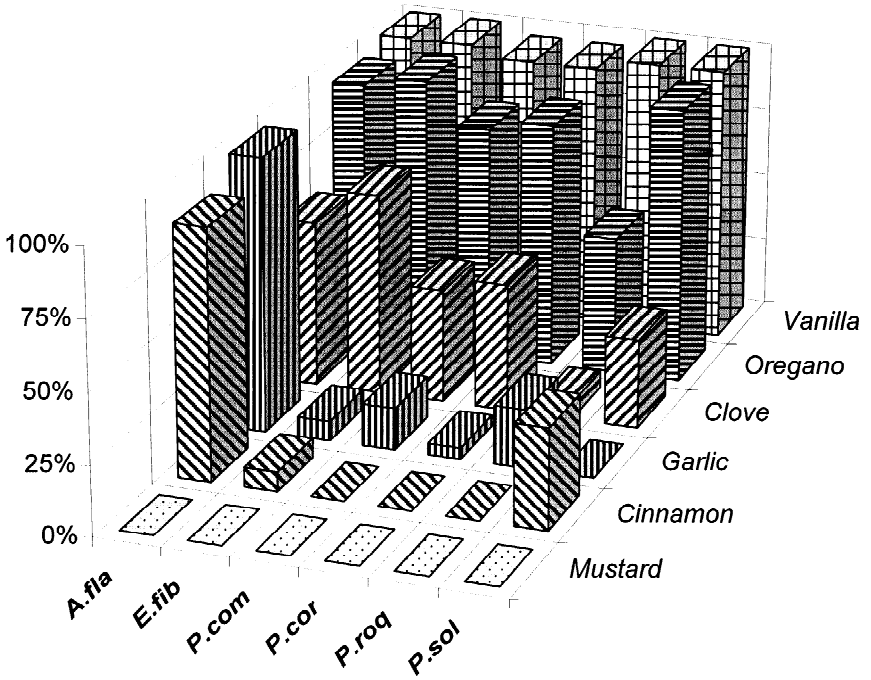

Fig. 2. Percent inhibition of common bread spoilage fungi (test b: *Aspergillus flavus* (*A.fla*), *Endomyces fibuliger* (*E.fib*), *Penicillium commune* (*P.com*), *P. corylophilum* (*P.cor*), *P. roqueforti* (*P.roq*) and *P. solitum* (*P.sol*)) incubated with 1 μl essential oil or oleoresins in a Petri dish system on CYA at 25°C in 7 days.

did not decrease growth, where as oregano OL, clove OL, garlic EO, cinnamon OL and mustard EO reduced growth with 50, 94, 79, 100, and 100%, respectively.

The growth curves, in Fig. 3, of *A. flavus* incubated with 1 μl of each compound, gives a representative picture of the standard deviation on the measurements of colony diameter. The deviations of the measurements are expressed as error bars. Both high and low standard deviations were observed. Since the three inoculation points of each plate were made in sequence the number of spores per point will decrease from the first to the third. Thus, high standard deviations indicates that the survival of the microorganisms are crucially dependent on the spore concentration in the inoculation

3.3. Minimal inhibitory concentration of AITC

The minimal inhibitory concentration (MIC) for mustard EO or allyl isothiocyanate (AITC) were determined for eight fungi and two yeast commonly found on bread (Table 1, test c). In Fig. 4, the relative growth after 7, 14 and 28 days as compared to a control are plotted for each microorganism at three different concentrations of AITC. The lowest concentration showed only slight inhibition after 7 days. At 0.5 μl AITC all moulds and yeast were inhibited by between 67 and 100%, whereas 1 μl AITC in the culture flask prevented growth of all organisms for more than 2 weeks.

These correspond to MIC values between 1.8 and 3.5 μg/ml gas phase for all the tested microorganisms. For *P. solitum*, *P. commune*, *P. corylophilum* and *P. discolor* MIC were 1.8 μg/ml gas phase. For *A. flavus*, *P. roqueforti*, *P. polonicum*, *P. palitans*, *E. fibuliger* and *Pichia anomala* the MIC values were 3.5 μg/ml gas phase. The real MIC values may be lower in vivo, since these experiment were performed with massive spore inoculations and the common natural contamination level is only a few spores.

Testing various microorganisms for the MIC values of AITC in the vapour phase Isshiki et al. (1992) reported that: for bacteria the MIC was between 34 and 110 ng/ml gas phase, for yeast

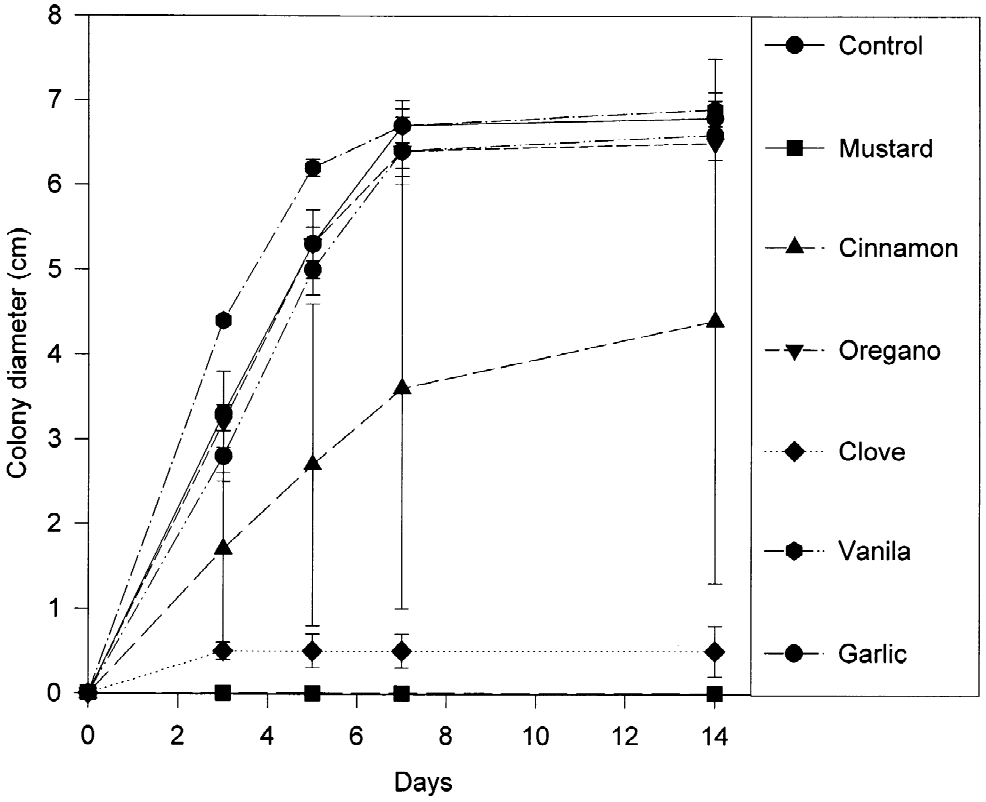

Fig. 3. Growth curve of *Aspergillus flavus*. Incubated with 1 μl of essential oils (mustard and garlic) or four oleoresins (cinnamon, oregano, clove and vanilla) on CYA at 25°C.

between 16 and 37 ng/ml gas phase and for moulds between 16 and 62 ng/ml gas phase. Similar to our experiment, MIC was defined as the concentration where there was no growth after 2 days for bacteria and after 7 days for yeast and moulds. Tsunoda (1994) on the contrary found concentrations between 3.8 and 118 μg/ml gas phase to inhibit fungi.

Delaquis and Mazza (1995) summarises previous results concerning the inhibitory effect of AITC: germination and growth of fungi were prevented at 20–40 μg/ml AITC in gas and 2 μl/ml when added to a liquid medium. Virtanen (1965) found MIC in liquid medium to be 2 μl/ml for fungi and 10 μg/ml for bacteria. Likewise Mari et al. (1993) reported that MIC for AITC on fungi was between 150 and 600 μg/ml liquid medium.

Sekiyama et al. (1994b) compared the effect of mustard extract (90% AITC) diluted in agar with the effect obtained exposing the extract to bacteria and fungi through the vapour phase. They concluded that the AITC were most effective exposed as a vapour directly to the microorganisms.

If the effect of AITC was only fungistatic growth would be expected after opening of the culture flasks after 14 days. In the lower part of Fig. 4 it is seen that only growth of *P. corylophilum* was still completely inhibited after 28 days at 0.5% AITC. Growth of *P. solitum*, *P. commune* and *E. fibuliger* was only decreased by 67, 55 and 35%, respectively. The rest of the microorganisms were only completely inhibited at 1 μl AITC. However, *P. roqueforti* and *A. flavus* had started to grow even at this concentration and were only retarded by 50 and 81%, respectively, after 28 days.

Like in the screening experiments, colonies cultured at concentrations close to MIC may have high standard deviations in colony diameter, as growth only occur in one or two of the three inoculation points. As the number of spores in each point decrease from the first to the third on each plate, the

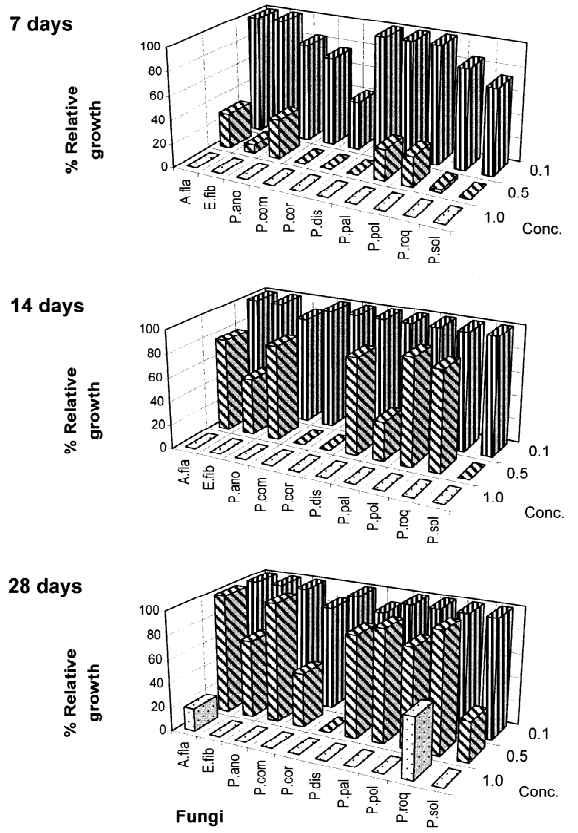

Fig. 4. Colony size of fungi (test c: *Aspergillus flavus* (*A.fla*), *Endomyces fibuliger* (*E.fib*), *Pichia anomala* (*P.ana*), *Penicillium commune* (*P.com*), *P. corylophilum* (*P.cor*), *P. discolor* (*P.dis*), *P. palitans* (*P.pal*), *P. polonicum* (*P.pol*), *P. roqueforti* (*P.roq*) and *P. solitum* (*P.sol*)) associated to bread relative to an untreated control. Graphs of 7 and 14 days shows the fungistatic effect of allyl isothiocyanate (AITC). After 14 days, the container was opened, whereas the graph at 28 days shows the fungicidal effect of AITC.

difference in response indicated that AITC can be both fungistatic and fungicidal depending on the concentration of spores and the concentration of AITC.

Summarised results showed that at 1.8 μg AITC/ml gas phase all microorganisms were strongly inhibited at least for 7 days. At higher concentrations AITC were more and more fungicidal. At 3.5 μg/ml gas phase AITC were fungicidal on all the microorganisms tested.

3.4. Sensory evaluation of active packaged bread

Principal component analysis (PCA) regression of the data from the sensorial evaluation of bread packed with AITC resulted in three principal components (PC) explaining 28.0, 23.3 and 20.7% of the variation in data, respectively. Thus the model explained 72.0% of the total variation in data. The first principal component only described the difference in sensory characters of rye and hot-dog breads. Whereas the effect of AITC concentration on sensory score as evaluated by the eight judges is described by PC2 and PC3. Fig. 5 shows a plot of PC2 against PC3, in this plot the sensory scores of the two bread types separates out in two groups. Hot-dog bread (as indicated with an h) in the top right corner, above the dotted line and rye bread (as indicated with an r) in the lower left corner. Packages with the highest concentration of AITC are located in the lower right corner of the plot. Comparing the two bread types it is evident that the threshold concentration of AITC is different. All rye bread packages containing up to 1 μl AITC (r0, r1 and r2) are grouped together and do not show a clear concentration effect, whereas the packages with 2 μl (r3) clearly separates out from the others. For hot-dog bread there is also a concentration effect, but some judges are able to sense AITC already at the lowest concentration (h1, 0.5 μl), while other judges only sense AITC when 1.0 or 2.0 μl is added. These results show that the sensory profile of packaged hot-dog bread is more sensitive to AITC than rye bread. This may be due to the acid flavour of rye bread, which have some resemblance to the pungent flavour of AITC at lower concentrations. Whereas the sweet flavour of hot-dog bread is more in contrast to the pungent flavour of AITC. Furthermore, hot-dog bread is more airy than rye bread and therefore probably retains more AITC. The sensory evaluation also indicated that the threshold for aroma change is lower than for taste. Although the panel of untrained judges gave different marks on the flavours they consistently had similar differences in marks between flavours.

In a different sensorial evaluation of bread, six slices of rye bread or four pieces of hot-dog bread were packed by a standardised method with AITC concentrations corresponding to MIC values found above. Results did not give a significant difference on taste or aroma at a 5% significant level. Sensorial judges did not identify the correct concentrations of AITC in a ranking test, suggesting that at lower AITC concentrations no significant differences are found on aroma and taste.

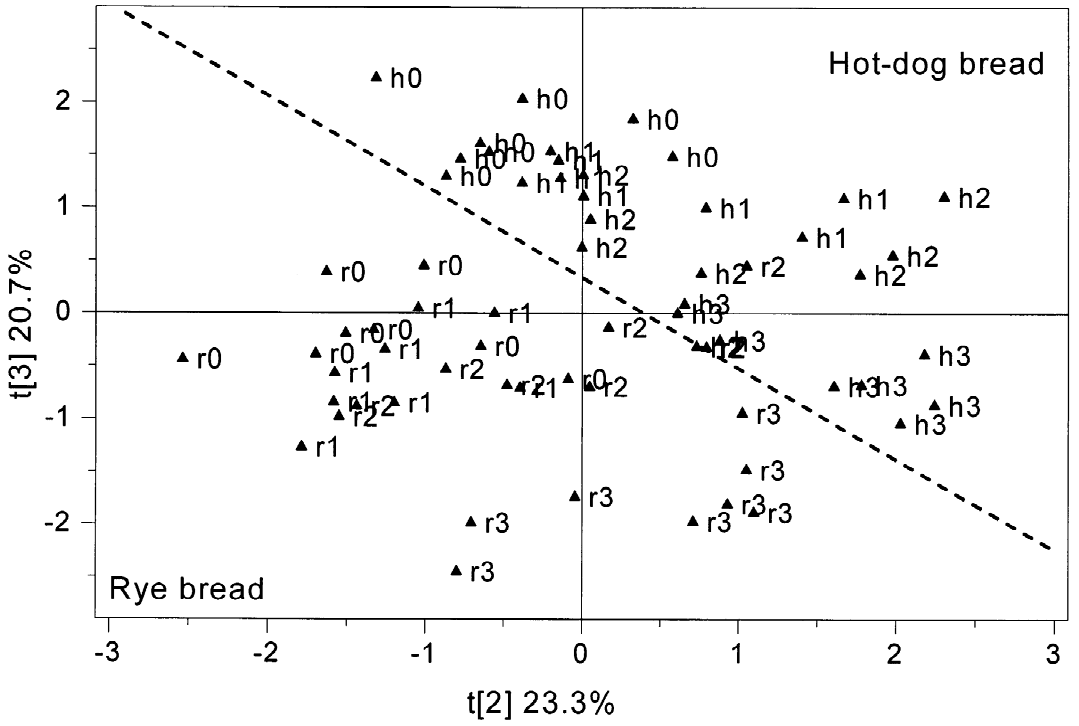

Fig. 5. Score plot from a principal component analysis of the sensory evaluation data of rye (r) and hot-dog (h) bread packaged with different concentrations of AITC, as indicated by the numbers. Control, 0.5, 1.0 and 2.0 μl AITC are labelled 0, 1, 2 and 3, respectively.

By estimation it was found that the minimal sensorial concentration for rye bread was higher than 2.4 μg AITC/ml gas phase in the packaged (the highest concentration tested). For hot-dog bread the minimal sensorial concentration was estimated to 1.8–3.5 μg AITC/ml gas phase. Taste was not influenced by the concentration of AITC, but marks given by the judges on the sweet aroma of hot-dog bread were lower at 3.5 μg AITC/ml gas phase.

Based on the sensory analysis and the MIC values for fungal growth rye bread is suitable for packaging in active packaging with AITC, whereas hot-dog bread is more likely to achieve a notable off-flavour. Nevertheless, it is expected that in real-scale packaging it would be possible.

The use of AITC still needs some more consideration due to its characteristics. AITC is described as pungent, penetrating, eye-watering, toxic and a flammable agent. It has been used as a flavour additive due to its characteristic aroma and taste and also in the 'negative' perfumery it has found use as a repellant. AITC appears on the FEMA/FRAS list but not recommended by the IFRA. Remarkably, the National Cancer Institute's Diet and Cancer branch studies the efficacy of AITC to prevent cancer in humans as it could possibly work as an anti-carcinogen in functional foods (Clark, 1992).

Despite all the studies with AITC and related compounds still more work is needed to explain the mechanism of action or degradation in organisms. It is suggested that AITC is degraded by several different pathways producing many different by-products. Therefore, the sum of the reactions taking place have a high antimicrobial activity on a broad spectrum of microorganisms. Therefore, before applying AITC, it should be considered how AITC might influence the production of extracellular metabolites which might be induced by AITC to an unwanted level.

One way of applying AITC could be to pack bread with a fungicidal concentration of AITC, but in a film permeable to AITC. After killing of the microorganisms AITC will migrate through the film, leaving the product sterile and with a concentration of AITC less than the sensory threshold. Sekiyama et al. (1995) showed that such films made of poly-

ethylene (PE) or ethylenevinyalcohol (EVA) had such characteristics.

The antimicrobial effects of AITC have been used to build a preservation system called WasaOuro® (Green Cross Corp., Osaka, Japan). Testing the effectiveness of WasaOuro, Worfel et al. (1997) discovered that the normal reproductive cycles of the stored grain insect pests *Lasioderma serricorne* and *Tribolinum confusum* were disrupted. The sensorial qualities of bread backed from flour packed and stored with WO pouches was nevertheless not different from an untreated control.

Several patents describe systems of applying the preservative effect of AITC, either as a spray (Sekiyama et al., 1994a) (US Patent nr.5334373), by a controlled-release (Fujita et al., 1995) or slow-release system (Chinuki et al., 1995)

In most European countries agents used in active packaging are considered as additives. At the moment there is no general law with regard to the use of allyl isothiocyanate as a preservatives, although general rules in Europe are under consideration. The substance have just been added to the 4th edition of the Council of Europe Blue Book on flavouring substances as a category A substance with a TDI of 0.06 mg/kg bw/day (Committee of Experts on Flavouring Substances, 1999).

4. Conclusion

Volatile antimicrobial substances from spices and herbs have proved efficient in the control of fungal spoilage by common bread spoiling fungi. Mustard essential oil, which primarily consists of allyl isothiocyanate (AITC) had the strongest effect, inhibiting all tested fungi for more than 2 week when applied at 1 μl per 250-ml culture flask. Cinnamon and garlic essential oils required slightly higher concentrations to prevent growth of all fungi completely. The sensory threshold was slightly higher than the minimal inhibitory concentration (MIC) for AITC on rye bread, thus the required shelf-life of rye bread could be achieved by active packaging with AITC. However, hot-dog bread may require the additional effect of other preserving factors to avoid off-flavour formation due to a slightly lower sensory threshold. The combination of several natural antimicrobials or alcohols and MAP may solve that problem. This is currently under investigation in our laboratory.

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