ABSORBENT PADS FOR FOOD TRAYS MADE FROM *SPHAGNUM* MOSS

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ABSTRACT

Absorbent pads were made from chlorite-treated (bleached) *Sphagnum papillosium* and placed on the top and bottom of Atlantic salmon (*Salmo salar*) individual fillet slices. Each fillet slice was packed in a high-density polyethylene tray in a modified atmosphere of 60:40 N_2/CO_2 to a gas:fillet slice volume ≈ 5:1 and stored at +4°C. These trays, and controls (fillet slices with a standard paper absorptive pad or no pad) packed in the same way, were independently removed from cold storage and sampled (n=3) at selected time intervals over a 30 day period. Tray gas composition, water content, water holding capacity, pH, colour, texture, smell, viable bacterial count, amount of soluble protein, amount of acid soluble peptides and free amino acid were assessed for each fillet slice. By the end of the experiment and compared to controls, the smell of the fillet slices stored with *Sphagnum* pads was deemed to be acceptable to a consumer. Between storage days 7-12 and 19-30 days in the stationary bacterial growth phases these same fillet slices had roughly half the viable bacterial counts per gram slice. No significant differences, between all fillet slices at each sampling interval, were found for any of the other parameters which were assessed, except for free amino acid content. These results show that absorbent pads made from *Sphagnum* moss may, with further development, have a commercial potential in that they can help extend the shelf-life and the quality of packaged fresh foods such as fish.

Key words: fish, food packaging, shelf-life

INTRODUCTION

Previous experiments have shown that *Sphagnum* moss bleached with chlorite displays microbiocidal properties (Børsheim *et al.*, 2001). When used as a packing material it had the ability to seemingly preserve and slow-down the on-set of putrefaction of whole Zebra fish for a number of days at room temperature and in the presence of oxygen (Børsheim *et al.*, 2001). Historical evidence for the preservative properties of *Sphagnum* moss is also abundant. Sterile *Sphagnum* moss bandages show excellent wound healing properties and their use dates back to the Bronze Age (Varley & Barnett, 1987). In particular they were extensively used in World War I (Riegler, 1989). *Sphagnum* moss is also used to preserve post-harvest fruit and vegetables, and even freshly caught fish (Riddervold, Pers. comm.). These functional properties of *Sphagnum* have been attributed to its remarkable ability to absorb liquid and to as yet an unidentified microbiocidal entity.

The aim of our current study is therefore to evaluate the use of chlorite-treated *Sphagnum* as a packaging material for fresh food stuffs. Fillet slices of salmon were selected for study because at present only 1 in 3 temperate marine fish is sold to the consumer (mostly export) as high-quality, high-profit fresh fish. If the shelf-life of fresh fish can be extended by only 20-30 % (2-3 days) without sacrificing sensory or nutritional quality (Declerck, 1976) then the economic and health benefits are potentially substantial. The salmon fillet slices were packaged in trays under modified atmosphere to mimic the present commercial standard. The only difference was that chlorite-treated *Sphagnum* was applied to the top and bottom of the fillet slice in the form of a pad made according to the ISO standard traditionally applied in the production of paper sheets from cellulose fibres and pulp. These trays, and controls (fillet slices with a standard paper absorptive pad or no pad) packed in the same way, were independently removed from storage at +4°C and sampled (n=3) at selected time intervals over a 30 day period. Tray gas composition, water content, water holding capacity, pH, colour, texture, smell, viable bacterial count, amount of soluble protein, amount of acid soluble peptides and free amino acid were assessed for each fillet slice.

MATERIALS AND METHODS

Pad production

66 *Sphagnum* sheets (12 x 24 x 0.1 cm) were produced to ISO standard 5269-1 using a custom-made sheet former, and a press (PTI, model 40140). Each sheet comprised 4 g of ground chlorite-treated whole *S. papillosum* plants (Ballance *et al.* In Press). Finally the sheets were cut down to 9.5 x 11 x 0.1 cm to form the pad.

Salmon packaging, storage and sampling

Fifteen salmon (*Salmo salar*) of 13-15 kg were obtained from a commercial fish farm. These were live-chilled, bled and gutted, and immediately iced prior to transport. All fish were manually filleted 3 days after slaughter to obtain fillet slices of 8 x 9 x 3 cm. One day later (defined as day 0) the fillet slices were individually packed in high-density 750 ml polyethylene trays and sealed with a polyethylene film in a modified atmosphere of 60:40 N_2/CO_2 to a gas:fillet slice volume $\approx$ 5:1. Three different sample series (treatments) were prepared: fillet slices packed without a pad, slices packed on their underside with a standard absorbent paper pad

currently used in food packaging, and fillet slices packaged with *Sphagnum* pads on the top and bottom side. In total 65 fillet slices were packed; 21 in each pad treatment and 23 without a pad. These were immediately stored at +4 °C. After 2, 6, 9, 13, 16, 20, 27 and 30 days three replicate fillet slices from each treatment were removed from cold storage for analysis. At day 0 only 2 fillet slices packed with no pad were sampled.

Analytical analysis (n=3) of sampled fillet slices

The O_2, CO_2 and N_2 content of the tray were quantified by a gas analyser. Muscle pH was measured in the fillet slices. Water content was determined by drying 2 g samples (n=3) of each fillet slice at 105 °C for 24 h. Water holding capacity was determined on ground muscle as described by Eide *et al.* (1982). Fillet slice colour was determined with a salmon graduate colour card. Extractable protein, acid soluble peptides and free amino acids were determined as previously described (Hultmann & Rustad, 2002; Hultmann & Rustad, 2004).

Microbiological analysis

A 50 g piece of fillet slice (n=1) was aseptically cut and homogenized at 200 rpm for 30 second in filter bags with 4 parts sterile peptone saline, pH 7.2, using a stomacher. These homogenates were further diluted (1:5) with peptone saline. Appropriate dilutions (10^{-2} – 10^{-6}) were spread on Long and Hammer agar plates (n=3) with 1% (w/v) added NaCl (van Spreekens, 1974) and incubated at 15 °C for 7 days, prior to counting the number of colony forming units (CFU) and expressing them per gram wet weight of fish (N.B. The water content of each fillet slice was relatively constant). Typical isolated colonies were identified by 16S rDNA sequence analysis (NCIMB Ltd, Aberdeen, Scotland).

Textural properties and smell analysis

Textural properties were measured with a TA.XT2 Texture Analyser as previously described (Hultmann & Rustad, 2002). Smell was determined by sensory analysis by a panel of 5-7 scientists each smelling the fillet slice immediately and 10 min after opening the package. Smell was ranked on a integer scale from 1 to 6 (1- fresh seaweed-like smell, acceptable, 2- odourless, acceptable, 3- slight fishy odour, acceptable, 4- significant fishy odour, borderline acceptable, 5- strong fishy odour, not acceptable, 6- totally off, i.e. putrid smell, not acceptable).

RESULTS AND DISCUSSION

The proportion of CO_2 in the atmosphere of all the packages (no significant differences between treatments) decreased from 60% at day 0 to a near constant value of around 44-45% after day 6. This is possibly because of diffusion of CO_2 into the fish muscle (Killeller, 1930). On the other hand the concentration of O_2 in the trays increased slightly from not detectable at the start of the experiment to a maximum of 0.18% by day 6. This is probably because of a slow diffusion of air into the tray. By day 9 no O_2 was detected in any tray because all is consumed, primarily by bacterial respiration. The pH and colour of all fillet slices remained constant at around 6 and salmon red respectively throughout the experiment. It is likely that the CO_2 content inside the tray had a buffering effect on fillet slice pH and a stabilising effect on the fillet slices pigments (Killeller, 1930).

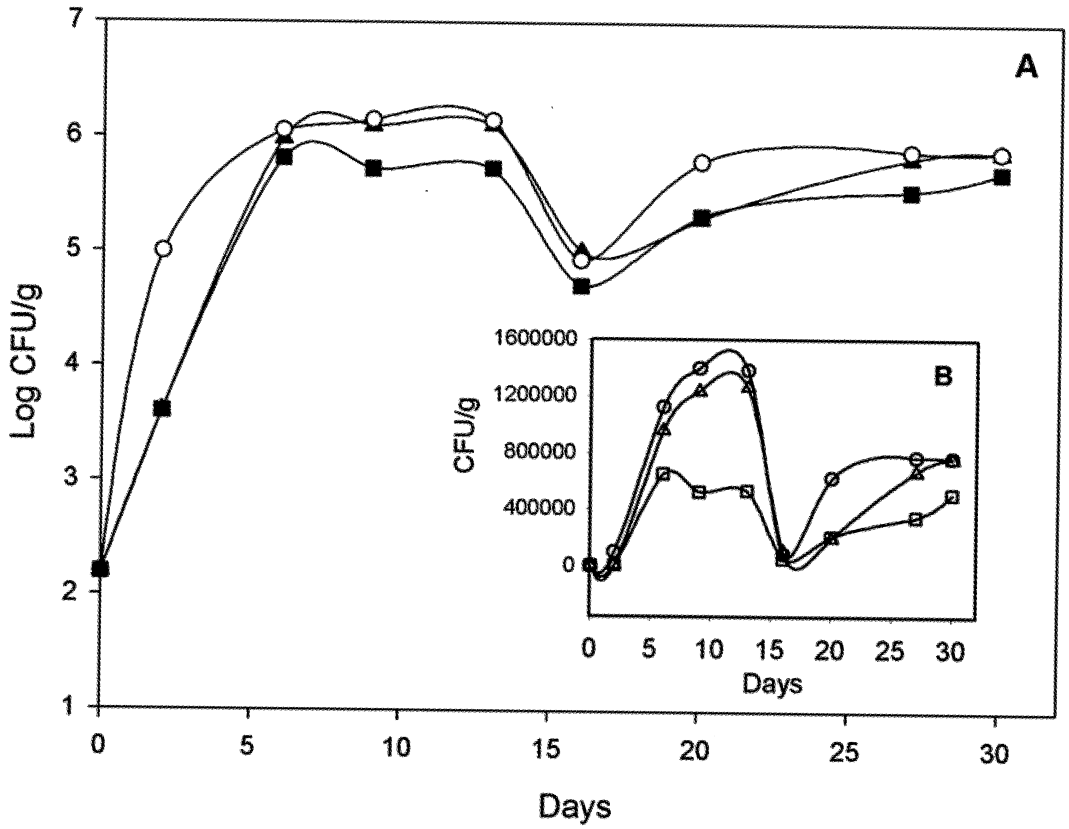

Fig. 1. Log (**A**) or number (**B**) of colony forming bacterial units (CFU) (mean, n=3 ±S.D.) per gram wet weight fillet slice packed without a pad (triangles), packed on the underside of the fillet slice with a standard absorbent paper pad (circles), and packed with *Sphagnum* pads (squares) on the top and bottom side. Fillet slices (n=1) were sampled on the time internals depicted.

Not surprisingly the modified atmosphere packaging (MAP) also had a large influence on the microbiology of the fillet slices over the course of the experiment. At the start of the experiment the observed viable bacterial count was about 10^2 CFU/g which increased at an exponential rate in the log-growth phase to 10^6 CFU/g after 6 days (Figure 1a). Such levels of viable bacteria are typical for MAP packed fish fillet slices (Reddy, 1997, Cann, 1984, Pastoriza, 1996, Stier, 1981). Although there is only one sampling point it is interesting to note that after 3 days storage in the log growth phase, fillet slices in contact with a standard pad used in current food packaging (Figure 1), had about a one and a half magnitude greater viable bacterial count (10^5) than fillet slices in the other treatments. The reason for this is currently unclear.

Between 6 and 12 days of storage the stationary growth phase became established were the viable bacterial count remained relatively constant (Figure 1a). The dominant bacterium in this growth phase was identified as *Photobacterium phosphoreum*; typical of MAP packaged fish (Dalgaard *et al.*, 1996). Between day 12 and 16 there is a death phase followed by a secondary log and stationary phase

(Figure 1a). *P. phosphoreum* remains the dominant bacteria in this growth phase, but in addition colonies of *Lactobacillus maltaromaticus* start to appear. In food microbiology it is usually standard practice to consider significant differences in bacterial growth in terms of orders of magnitude. In this case no marked difference is seen in the stationary growth phases between fillet slices in each of the three treatments. However, examination of Figure 1b reveals that the viable count of bacteria in the fillet slices treated with the *Sphagnum* pad, in both the first and second stationary growth phases, is consistently about 50% less than the other control treatments. It therefore seems clear that the presence of such a pad has some influence in terms of decreasing the overall maximum viable bacterial count of the fillet slices. A number of explanations are possible: 1. the presence of the extra *Sphagnum* pad on the top of the fillet slice, 2. interaction of the pad with the fillet slice or with the bacteria (see Painter, 2003). 3. the *Sphagnum* pad has some anti-microbial properties in which the division of absorbed bacteria is impeded.

In addition to these properties we also found that the smell of the fillet slices stored with the *Sphagnum* pads were deemed acceptable to a consumer, in contrast to control fillet slices, right up to the end of the experiment. All fillet slices that contained pads had no free liquid floating around in the trays because as expected all was absorbed by the pad. Nevertheless, pad treatment had no significant effect on fillet slice texture, water content, water holding capacity, extractable protein or acid extractable peptides. The content, however, of extractable free amino acids was significantly different.

In conclusion it is clear to see that the application of *Sphagnum* pads to foods or food packaging may have a commercial potential. Further research, however, is required in designing a more durable pad and investigating the true extent of it apparent antimicrobial properties and how these may be optimised.

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REFERENCES

Ballance S., Børsheim K.Y., Inngjerdingen K., Paulsen B.S. & Christensen B.E. (In Press). A re-examination and partial characterisation of polysaccharides released by the mild acid hydrolysis from the chlorite-treated leaves of *Sphagnum papillosum*. *Carbohydrate Polymers*.

Børsheim K.Y., Christensen B.E. & Painter T.J. (2001). Preservation of fish by embedment in *Sphagnum* moss, peat or holocellulose: experimental proof of the oxopolysaccharidic nature of the preservative substance and of its antimicrobial and tanning action. *Innovative Food Science & Emerging Technologies* **2**, 63-74.

Dalgaard P., Meljlholm O. and Huss H.H. (1996). Conductance methods for qualntitative determination of *Photobacterium phosphoreum* in fish products. *Journal of Applied Bacteriology* **81**, 57-74.

Declerck D. (1976). Organoleptic, chemical and microbiological aspects of vacuum packed and unpacked smoked pink salmon. *Mededeling van het Rijksstation voor Zeevisserij* **113**, 1-9.

Eide O., Børresen T., Strøm T. (1982). Minced fish production from capelin (*Mallotus villosus*). A new method for gutting, skinning and removal of fat from fatty fish species. *Journal of Food Science*. **47**, 347-349, 354.

Hultmann L. & Rustad T. (2002). Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). *Journal of Aquatic Food Product Technology* **11**, 105–123.

Hultmann L. & Rustad T. (2004). Iced storage of Atlantic salmon (*Salmo salar*) – effects on endogenous enzymes and their impact on muscle proteins and texture. *Food Chemistry* **87**, 31-41.

Killeller S.M. (1930). Carbon dioxide preservation of meat and fish. *Industrial and Engineering Chemistry* **22**, 140-143.

Painter T.J. (2003). Concerning the wound-healing properties of *Sphagnum* holocellulose: the Maillard reaction in pharmacology. *Journal of Ethnopharmacology* **88,** 145-148.

Pastoriza L., Sampedro G., Herrera J.J. & Cabo M.L. (1996). Effect of carbon dioxide atmosphere on microbial growth and quality of salmon slices. *Journal of the Science of Food and Agriculture* **72**, 348-352.

Reddy N.R., Solomon H.M., Yep H., Roman M.G. & Rhodehamel E.J. (1997). Shelf life and toxin development by *Clostridium botulinum* during storage of modified-atmosphere-packaged fresh aqua cultured salmon fillet slices. *Journal of Food Protection* **60**, 1055-1063.

Riegler N.N. (1989). *Sphagnum* moss in World War I: The making of surgical dressings by volunteers in Toronto, Canada, 1917-1918. *Canadian Bulletin of Medical History* **6**, 27-43.

Stier R.F., Bell L., Ito K.A. (1981). Effect of modified atmosphere storage on *C. botulinum* toxigenesis and the spoilage microflora of salmon fillet slices. *Journal of Food Science* **46**, 1639-1642.

Van Spreekens K.J.A. (1974). The suitability of modification of Long and Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. *Archiv fur Lebensmittelhygiene* **25**, 213–219.

Varley S.J. & Barnett S.E. (1987). *Sphagnum* moss and wound healing I. *Clinical Rehabilitation* **1**, 147-152.