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FINAL REPORT

Project Armis No. 4196

# Extending the Shelf Life of Fresh Sliced Mushrooms



The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 2

Contents

# EXTENDING THE SHELF LIFE OF FRESH SLICED MUSHROOMS

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## SUMMARY

The Irish mushroom industry is expanding rapidly as is the demand for sliced mushrooms. To increase the competitiveness of Irish mushrooms for export their shelf life should be extended to compensate for the time lost in transit. The aim of this project was to extend the shelf life of sliced mushrooms by 50% using novel processing treatments and/or packaging. A method was established to assess the effects of different treatments on mushroom quality. This method was followed using solutions of citric acid, hydrogen peroxide, EDTA, nisin, diacetyl, vitamin E, ascorbic acid, rosemary extracts and sodium metabisulphite.

Treatments with 40 g/l citric acid, 5% hydrogen peroxide or 40 g/l EDTA were very effective in extending the shelf life of sliced mushrooms. When a soaking period of 10 min was used these three treatments improved the shelf life of the sliced mushrooms by about 50% when compared to control mushrooms soaked in water. Effectiveness of the treatments varied from batch to batch of mushrooms and was found to be linked to mushroom shear value with the toughest mushrooms responding least favourably to the treatments. Sulphites have been used by the mushroom industry for a long time but treatment with 1 g/l sodium metabisulphite for 10 min was found to have a poor effect on mushroom quality.

The novel packaging treatments investigated, modified atmosphere packaging and using absorbent inserts, were ineffective. Mushrooms have a very high respiration rate and so modified atmosphere packaging is unsuitable because mushrooms rapidly use up all the available oxygen which leads to bad flavours and the risk of growth of *Clostridium botulinum*.

The final part of this report describes a dried, flavoured mushroom snack, with a long shelf life, which was developed for the export market.

# INTRODUCTION

## The mushroom industry

The mushroom industry in Ireland has developed phenomenally over the past decade. A sevenfold increase in mushroom output since the early 1980s has been reported, with a current value of £70 million. Most of the mushrooms are exported to the UK; in 1997 the value of the exports was estimated at £55 million. Irish mushrooms compete in UK supermarkets with UK and Dutch mushrooms, but Ireland is now the biggest exporter of fresh mushrooms to the UK, filling 50% of the supermarket demand, and 20% of the overall demand. To improve exports yet further, the Irish industry must increase sales to UK food processing companies. Many of these companies, such as those manufacturing pizzas and pies require sliced or diced mushrooms. To compete favourably alongside UK sliced mushrooms Irish slices need a longer shelf life to make up for the time lost in transit.

Consumer demand for ready-to-use foods has rapidly increased in recent years. Pizza and pie producing companies have a high demand for sliced mushrooms and supermarkets are selling an increasing number of small packs of sliced fresh mushrooms. The mushroom industry supplies about 5 to 25% of its fresh output as slices. Diced mushrooms are also supplied, but to a lesser extent.

## Mushroom spoilage

All fresh mushrooms are prone to spoilage; this is particularly true for sliced or diced mushrooms. Slicing creates a larger surface area which amplifies the spoilage problems. Mushroom spoilage mechanisms include dehydration, enzymatic browning and bacterial growth. Mushrooms have a shorter shelf life than most ready-to-use vegetables because their respiration rate is rapid and they have no barrier to protect them from water loss or from microbial attack. Enzymatic browning occurs when the enzyme, tyrosinase, makes contact with its substrate and initiates a series of reactions which produces brown melanin pigments. Contact between the enzyme and its substrate can occur when mushrooms are bruised, cut, or damaged by microbial growth.

Microbial spoilage of mushrooms is usually due to the growth of pseudomonad bacteria. As these bacteria grow, they break down the mushroom fibres which softens the mushroom and leads to enzymatic browning. The major species responsible for this is *Pseudomonas tolaasii* which produces a toxin that lyses mushroom cells. The resulting brown pigments and surface lesions are symptoms of the disease, "bacterial blotch". Growth of pseudomonad bacteria also causes slime to form on the mushroom surface. Chilled storage (4°C) of mushrooms from harvest to cooking helps to maintain good quality (Gormley, 1975) by reducing the rate of bacterial growth and enzyme activity. However, in this project, the shelf life of sliced mushrooms was extended further by introducing novel processing treatments.

## Brown mushrooms

In all the experiments described in this report white strains of the common mushroom, *Agaricus bisporus*, were used. A brown strain of *Agaricus bisporus* was also tested for some of the treatments. Brown strains, called "chestnut mushrooms" or "Paris browns", have brown skins but their flesh is creamish white and so when sliced they exhibit similar shelf life problems to white strains. The response of brown strains to the treatments was similar to that of white strains.

## Mushroom production

Mushroom crops develop in a series of flushes in weekly cycles. About 70% of the yield comes from the first two flushes. First and second flushes are harvested over four days, with most being picked on the third day. Mushrooms from flush one, two and three were tested in this project to see if there were any differences in their shelf life and their response to treatments. A comparison was also made of mushrooms grown on phase II and phase III compost. Phase III compost is compost that has been taken through phase I - wetting and stacking of raw materials, and phase II - pasteurisation and conditioning, and is then inoculated and colonised with spawn. The colonisation involves 14 - 15 days incubation at 25°C. The

substrate is then milled and transferred to bags. Phase III compost, the end product, is a fully spawn-run compost, delivered to the grower and ready to case immediately after stacking out. Phase III compost has only been available in Ireland since 1994 and in early 1997 it only accounted for 7% of the compost market, but it has been shown to improve yields and reduce crop cycle time. Phase II compost is bagged after pasteurisation and conditioning, and must be spawned and incubated by the mushroom grower. Within the Irish mushroom industry there is some debate over whether phase II or phase III compost is best. The pros and cons of each were discussed at the Cross Border Mushroom Conference in 1997 and are reported in the conference proceedings (Kilpatrick, 1997). As with any new process there have been some teething problems but as time progresses the advantages of phase III compost will increasingly outweigh the disadvantages. Certainly, it has been popular in the Netherlands where, in 1997, 60% of the compost used was phase III.

Only post-harvest mushroom treatments were considered, in this project, to improve mushroom quality, however, quality can also be affected during mushroom production. Some approaches to this are being investigated at the Teagasc research centre at Kinsealy (Connolly, 1997).

## Safety aspects

Cases of illness from pathogenic contamination of mushrooms are rare. To minimise the risk of this, correct pasteurisation of compost should be ensured, irrigation water should be uncontaminated and strict hygiene rules, such as the wearing of gloves, should be adhered to by mushroom handlers. Botulism has been linked to mushrooms on a few occasions but the causative agent, *Clostridium botulinum*, does not grow in the presence of oxygen and the toxin does not form at refrigeration temperatures (Sugiyama, 1982). *Campylobacter* has also been linked to mushrooms but these pathogenic bacteria are killed by pasteurisation and although they can survive at 4°C they do not grow below 28°C.

The effects of an extended shelf life on food safety should be borne in mind. For example, poor hygiene might cause contamination with pathogenic bacteria and an extended shelf life might then lead to the pathogens growing

to dangerous levels. Safety aspects of the treatment solutions are also very important and are included in later sections of this report. Indeed, the safety concerns about the current use of sulphites partly inspired this project.

## Report outline

This report details the standard methods used to measure mushroom quality and describes those treatments which were found to extend the shelf life of sliced mushrooms, followed by treatments which were found to have little or no effect on mushroom keeping quality. Finally, the development of a new mushroom snack product with an extended shelf life is described.

# METHODS USED TO ASSESS EFFECTIVENESS OF MUSHROOM TREATMENTS

Consumers, supermarkets and processing companies demand white, firm mushrooms of good flavour. However, appearance is the most important criteria for influencing purchase. In Ireland and the UK the whitest mushrooms generally fetch the highest prices.

## Colour

The surface colour of sliced mushrooms was measured with a Minolta Chroma Meter, model CR-331 (Fig. 1), using the Hunter Lab colour scale. This instrument defines colour numerically in terms of its lightness or "L" value (0 = black, 100 = white), "a" value (greenness 0 to -100, redness 0 to +100) and "b" value (blueness 0 to -100, yellowness 0 to +100). Typical "L" values for the caps of white strain mushrooms are 92-95 when measured within 24 hours

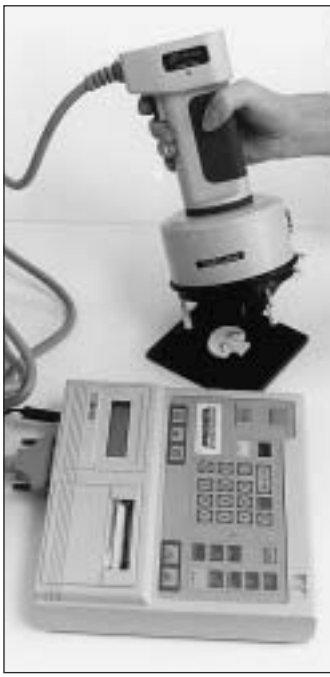


***Fig. 1:*** *Measuring mushroom colour*

from harvest, for sliced mushrooms the values usually range between 80 and 88. To measure the colour of a mushroom slice, it was placed flat on a black tile and the Chroma meter's measuring head was placed over as much mushroom as possible. The measuring surface included the cap, gills and stipe. The mean (average) and standard deviation for the colour of 10 slices were calculated.

## Texture

▲ ***Fig. 2:*** *Measuring mushroom toughness*

The texture of the mushroom slices was determined using a T-2000 Texture Test System (Kramer design) with a standard shear compression cell (model CS-1) (Fig. 2). After calibrating the instrument, 50 g of the sliced mushrooms were placed into the sample cell. The force required to shear the mushrooms was recorded. The larger the force, the tougher the mushrooms were. A second sample of 50 g was also tested and the mean of the two results was calculated. Typical shear values for fresh (<24 h) mushrooms are 900 - 1000 N/ 50 g.

## Microbiology

Microbial spoilage was monitored by recording the number of slices on which one or more slimy patches of bacterial growth could be seen, by eye. The number of pseudomonad bacteria on the mushrooms was also determined by mixing a mushroom sample with diluent and adding it to agar (Oxoid CM559 plus SR103E) plates on which only pseudomonad bacteria could grow. The plates were incubated at 25°C for 48 h and then the bacterial colonies were counted to determine the number of colony forming units per gram (cfu/g) of mushroom.

## Development of treatment methods

Four methods of applying the treatment solutions were tested. Spraying, brushing, and soaking sliced mushrooms all resulted in too much water absorption which increased microbial spoilage; soaking whole mushrooms was found to be the best method. Several concentrations of the substances were tested with different soak times ranging from 5 to 15 min. It was anticipated that soaking treatments should be as brief as possible. The optimum soak time of 10 min for hydrogen peroxide treatment of mushrooms was unexpected. To test the effect of the 10 minute soaking period, the standard method for assessing treatments was followed using soaking periods in chilled distilled water of 2 and 10 min. The mushrooms which had been soaked for 2 min maintained their white colour slightly better than the mushrooms soaked for 10 min, but the pseudomonad count was similar for both soaking treatments, and visual inspections showed bacterial spoilage to be similar for both treatments or sometimes more evident for the 2 min than for the 10 min soaked mushrooms. In view of these results, 10 min soaking periods were used for treated and control mushrooms.

## Standard method for assessing treatments

Unwashed, whole white mushrooms *(Agaricus bisporus*), with caps of about 5 cm diameter, were supplied from the desired flush and phase. After picking they were kept chilled (4°C) for up to 24 hours. The mushrooms were soaked in the treatment solutions at the given concentrations, or in distilled water (control) for 10 minutes and then placed on absorbent paper to remove excess surface water. The mushrooms were sliced to a width of 3 mm in a food processor and then spread on absorbent paper (Fig. 3). Six complete vertical cross-sections through the cap and stipe, from different mushrooms, were placed, slightly overlapping, along the length of a transparent plastic food tray. Vertical cross-section slices with little or no stipe were spread over the rest of the tray to provide a total of 60 g mushrooms (Fig. 4). In every experiment, eight to ten packs were prepared for each treatment, as required. In addition, two packs of 50 g of slices were

***Fig. 3:*** *Soaking and slicing mushroooms*

***Fig. 4:*** *A mushroom pack*

prepared for both the treated and control mushrooms, for microbiological tests. The packs were overwrapped with perforated film (XSC MPF Filmco, Viskase (UK) Ltd.), then weighed and finally stored at 4°C.

On day 0 the mushrooms in two control packs were tested for colour and texture and the mushrooms from the packs designated for microbiology were analysed for pseudomonads.

The mushrooms were stored at 4°C for up to 19 days and monitored at intervals for signs of deterioration. On each test day, two packs for the treated and for the control mushrooms were weighed so that the mass loss through the film could be determined. Six slices from each pack were examined for microbial spoilage. The mushroom slices were transferred to a clean tray and weighed. Colour and texture measurements were made and pseudomonads were enumerated.

## TREATMENTS WHICH EXTENDED MUSHROOM SHELF LIFE

Treatments which can extend the shelf life of fresh sliced mushrooms must reduce bacterial growth, enzymatic browning, or mushroom metabolism. An ideal treatment would inhibit all three spoilage mechanisms. In an attempt to find a treatment that could improve mushroom shelf life, several antimicrobial and antioxidant substances were screened using the standard method for assessing treatments. This method compares the effects of the treatment to the effects of water. Obviously, to be accepted by the mushroom industry new treatments must be better than those treatments already used in industry. Some mushroom growers think that sulphites are very effective at whitening mushrooms, but due to the "bad press" sulphite has received, they are keen to replace it. To compare the effectiveness of novel treatments with the existing treatment, sodium metabisulphite was also tested. Its lack of activity (see later) was unexpected; had it been anticipated, then assessment of stabilised chorine dioxide (Oxine/Puragene), would also have been carried out. Chlorine dioxide is increasingly being used by mushroom processors. A research group in the USA (Beelman, 1987)

found that Oxine was "very effective in controlling bacterial growth and colour deterioration when used at a level of 50 ppm or higher with a two minute or longer wash period at about 12°C". The activity of chlorine dioxide is due primarily to oxidation, not chlorination, which means that few, if any, chlorinated organic compounds are produced. The suppliers of Puragene (Vernagene, Bolton, UK) state that it has up to five times the disinfection power of chlorine, it is non-corrosive (at the concentrations used) and is unaffected by pH.

Three novel treatments were found to be very effective at increasing the shelf life of mushrooms and these are discussed below.

## The effects of citric acid, EDTA and hydrogen peroxide on mushroom shelf life

Citric acid is widely used as an additive in the food industry. It is relatively cheap and is safe. The maximum concentration of citric acid permitted in foods is *quantum satis*, *i.e.* unspecified, but not higher than that necessary to achieve the intended purpose (European Communities Regulations 1997 on Control of Additives for use in Foodstuffs). Citric acid is supplied as a powder which is soluble in water to form an acidic solution. There are several mechanisms by which citric acid might reduce mushroom spoilage. All organic acids are antimicrobial by virtue of their low pH and also by dissociation of the acid molecule within bacterial cells. Citric acid is also a metal chelator: metal ions are necessary for bacterial growth and for enzymatic browning reactions but citric acid traps the metal ions and makes them unavailable. In the work reported here, solutions of 40 g/l citric acid in distilled water were used. Preliminary trials showed that treatments with solutions of 20 g/l citric acid also reduced mushroom spoilage but were less effective than treatments with 40 g/l citric acid.

Ethylenediaminetetraacetic acid (EDTA) is a white powder which is soluble in water. It has the American GRAS (generally regarded as safe) status and calcium disodium EDTA is permitted in a variety of foods up to a maximum of 75 or 250 mg/kg, depending on the food (European Communities Regulations 1997 on Control of Additives for use in Foodstuffs). Like citric

acid, EDTA is acidic and is a metal chelator and so it has the potential to inhibit microbial growth and enzymatic browning. Solutions of 40 g/l EDTA were used to treat the mushrooms but it is likely that lower concentrations would also have been effective.

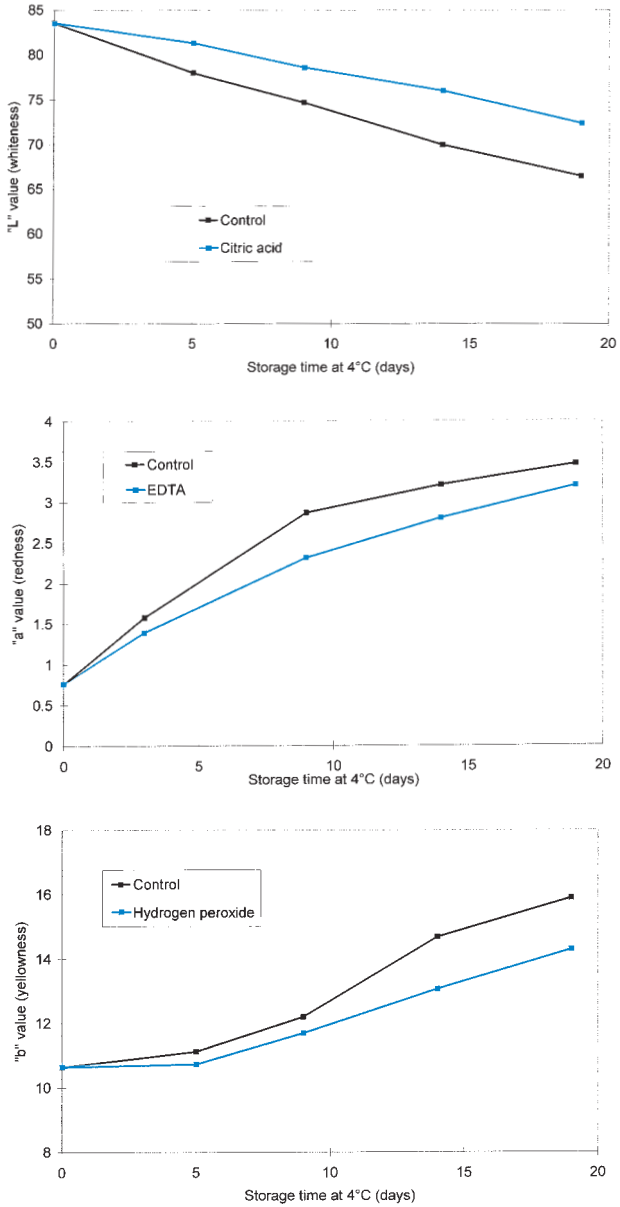
Hydrogen peroxide is a colourless liquid which is soluble in water. It does not appear in the European Communities Regulations 1997 on Control of Additives for use in Foodstuffs, however it is found naturally in many foods as a result of microbial metabolism. Its antimicrobial properties have been known for many years and it has been widely used as a sterilant for aseptic packaging. In the USA hydrogen peroxide is an approved bactericide for some dairy products and is used for disinfecting fruit and vegetables (Juven and Pierson, 1996). Its activity is due to its oxidising effects on bacteria and it also bleaches mushrooms during the soaking period. A 5% (v/v) solution was used in the experiments reported below.

### Effect of storage and treatments on mushroom colour

As mushrooms age they loose their whiteness and turn brown (see introduction). Brown coloration is uneven because it occurs where mushroom cells are damaged. For each mushroom the colour recorded was the average of an area of 5 $cm^2$ and each value plotted on charts was an average of ten slices. Hunter "L" values for ten slices had standard deviations of 2 to 6 units and the ranges (minimum to maximum values) were from 5 to 20 units.

Treatment with citric acid, EDTA or hydrogen peroxide usually slowed the rate of this colour change. The effectiveness of the treatments varied from batch to batch of mushrooms: sometimes the treatments were extremely effective, but occasionally they had little effect (see later). Figure 5 shows results from one experiment for each of the three treatments for Hunter "L", "a" and "b" values.

An increase in Hunter "a" and "b" values is always accompanied by a decrease in L value and so from here on only whiteness (L) values will be given.

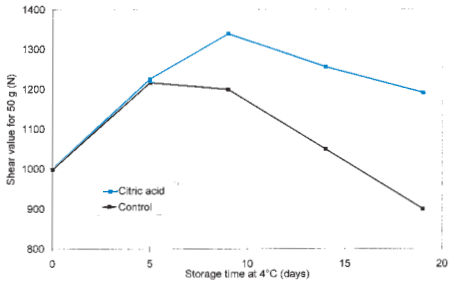



▲ ***Fig. 5 a, b and c:*** *Effect of citric acid, EDTA and hydrogen peroxide treatments on whiteness, redness and yellowness of mushrooms stored at 4°C*

## Effect of storage and treatments on mushroom shear values

For approximately five days after harvesting the texture of mushrooms became tougher. This toughening was followed by a softening phase which might be related to the breakdown in the mushroom tissue caused by bacteria. The effect of the citric acid, EDTA and hydrogen peroxide treatments was to reduce the rate of the softening phase (Fig. 6).

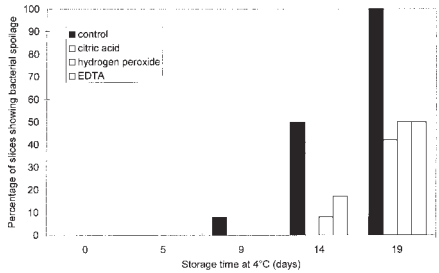
## Effect of storage and treatments on microbial spoilage of mushrooms




▲ ***Fig. 6:*** *Effect of treatment on texture of mushrooms stored at 4°C*

Visual signs of microbial growth on mushroom slices were not usually seen until after nine days of storage. The percentage of slices showing spoilage increased thereafter. All three treatments reduced the rate of microbial spoilage of the mushrooms (Fig. 7).

The number of pseudomonad bacteria on control mushrooms on day 0 was around one million cfu/g. The treatments reduced the number of pseudomonad bacteria on the mushrooms, often by as much as 100 to 1000

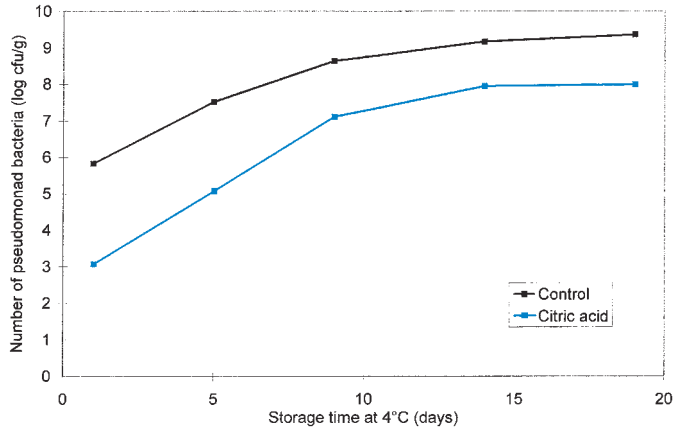



▲ ***Fig. 7:*** *Effect of treatments on bacterial spoilage of mushrooms stored at 4°C*

- fold, and this difference was usually maintained throughout storage as the number of pseudomonad bacteria increased (Fig. 8).

### Effect of flush number on mushroom quality

Mushrooms of strain L501, grown on phase III compost, from flushes one,




▲ ***Fig. 8:*** *Effect of treatment on number of pseudomonad bacteria present on mushrooms stored at 4°C*

two and three were monitored over 18 days of chilled storage. Flush two mushrooms were whiter, tougher and showed less bacterial spoilage than flush one and flush three mushrooms. The experiments were repeated with Silvan strain, but mushrooms from all three flushes were of similar whiteness on day 0 (Hunter L value of 93-95 for whole mushrooms) and the keeping quality was similar for all three flushes. Shear values, however, did vary, flush one were the toughest (1010 N/50 g), then flush two (980 N/50 g) and flush three were the least tough (870 N/50 g). Likewise, for Silvan mushrooms grown on phase II compost, flush one were the toughest and flush three were the least tough.

Data analysis showed a correlation between initial mushroom shear value and the effectiveness of the citric acid, EDTA and hydrogen peroxide treatments. Because a link was found between shear value and flush number

there was also an indirect correlation between the effectiveness of the treatments and flush number. It follows, therefore that the treatments were most effective for flush 3 mushrooms.

### Effect of compost phase on mushroom quality

No difference in mushroom whiteness was found for freshly harvested (Hunter L value of 93-95 for whole phase III mushrooms and 94 for phase II) or stored control mushrooms from each type of compost. However, phase III grown mushrooms were consistently less tough than phase II mushrooms (*e.g.* 990 N/50 g versus 1050 N/50 g, respectively) and treatments were most effective with least tough mushrooms.

### Effect of citric acid treatment on flavour

An experiment was carried out using taste panellists to see if the citric acid treatment gave rise to any off-flavours. The citric acid concentration in the treated mushrooms was not measured (a method is available from the British Standards Institution), but it would be considerably lower than that of the soaking solution (40 g/l).

Fresh mushrooms were soaked for 10 min in 0 (control) or 40 g/l citric acid. The mushrooms were sliced (3 mm) then packed (as standard) and stored overnight at 4°C. The slices were cooked in a microwave oven with butter or a low fat garlic oil spray. Twenty taste panellists were presented with control and citric acid treated mushroom slices and asked which sample they preferred and why. Nine tasters preferred the citric acid treated mushrooms and none of the tasters recorded noticing an acidic taste; this indicated that the citric acid was undetected by the panel.

### Discussion on the effectiveness of the treatments

Treatments with citric acid, EDTA and hydrogen peroxide were all effective in maintaining good mushroom quality for extended storage times. It is not

easy to pick out the best treatment because of the large amount of variation found between experiments and because more than one quality parameter was monitored. It is possible that effectiveness is proportional to the concentration of the treatment solution and so small adjustments in concentration could make all three treatments equally effective. The choice then would be down to cost of the required amount of substance. Safety is an important issue too - of the three substances, citric acid is probably the most widely used in foods and current legislation supports its use in preference to EDTA or hydrogen peroxide. This is why only citric acid treated mushrooms were used for the taste trials. The inability of the taste panel to distinguish between citric acid treated and control mushrooms further supports the application of citric acid as a treatment. Citric acid and EDTA are easier to handle than hydrogen peroxide, as they are not liquids, they do not deteriorate with age and are not corrosive. An advantage of hydrogen peroxide, however, is that it can whiten mushrooms on contact, in contrast, citric acid causes a slight yellowing. Fortunately it is only the surface which becomes slightly yellow and so when the mushrooms are sliced it is hardly noticeable.

The experimental method used to assess different treatments was designed to monitor changes in colour, shear value, mass loss, microbial spoilage and number of pseudomonad bacteria over time in chilled storage. It was not designed to quantify shelf life. There are no general specifications available from the Irish mushroom industry, which describe the quality of mushrooms at the end of their shelf life. However, to summarise the data collected in this research project and to conclude whether the project objective (to extend shelf life by 50%) was achieved, it was necessary to quantify shelf life.

Colour is the most obvious indicator of quality to the consumer - it relates to the age of the mushrooms, handling, and microbial spoilage - and so colour alone was used as an indicator to quantify shelf life. An experiment was set up to record the colour of commercially prepared sliced mushrooms at the end of their “use by” date. Four packs of mushrooms, each containing 280 g of slices, were purchased from a local supermarket. They were refrigerated at 4°C until their “use by” date, which was 5 days after they were first put on display in the supermarket. The colour of 30 slices from

each pack was measured with the Chroma meter. The procedure was repeated with four more packs of mushrooms which were prepared by the same company on a different day. The average whiteness (Hunter "L") value for the 240 slices was 76, with a standard deviation of 5.4. To quantify the shelf life of the control and treated mushrooms this value was used as a specification. The shelf life of laboratory prepared sliced mushrooms was taken as the number of days the sliced mushrooms could be stored at 4°C before their colour dropped to a Hunter "L" value of 76. This procedure is not ideal as it is only based on a decision made by one supermarket and /or sliced mushroom supplier. An alternative approach would be to present sliced mushrooms, showing different extents of browning, to a large number of potential buyers and ask which mushrooms would be consumed. The colour of these could be measured and the average value used as the specification, in the same way. Neither approach is ideal as they do not take into account the texture and flavour of mushrooms, nevertheless, it is a useful index or marker by which to quantify shelf life.

Table 1 shows shelf life for control and treated mushrooms calculated in this way. It can be seen from the minimum and maximum values that the effect of treatments on mushroom shelf life varied, quite considerably, from batch to batch of mushrooms. For citric acid treated mushrooms the extension in shelf life was, on average, 50%. It was slightly less for the EDTA and hydrogen peroxide treatments, but it should be noted that less experiments were carried out with EDTA than with citric acid or hydrogen peroxide. The effectiveness of all the treatments was linked to the initial texture of the mushrooms: less tough mushrooms responded better to the treatments than did tougher mushrooms. An explanation for this has not been established but it is speculated that it is due to different uptake rates - it may be easier for treatment solutions to spread through less tough mushrooms as they might have a looser cellular structure than tougher mushrooms.

**Table 1:** The shelf life* for batches of sliced mushroom stored at 4°C

| Treatment | Number of experiments | Minimum shelf life (days) | Maximum shelf life (days) | Mean shelf life (days) |
|---|---|---|---|---|
| Control | 16 | 6 | 14 | 8 |
| Citric acid (40 g/l) | 12 | 10 | 16 | 12 |
| EDTA (40 g/l) | 4 | 8 | 15 | 10 |
| Hydrogen peroxide (5% v/v) | 12 | 9 | 15 | 11 |

*days for L value to reach 76

The effectiveness of the treatments is illustrated in the photographs (Fig. 9). The mushrooms were third flush Silvan strain from phase III compost and they were treated using standard procedures.


▲ ***Fig. 9:*** *Mushrooms after 14 days storage at 4°C; treatments from left to right: 40 g/l citric acid, 40 g/l EDTA, 5% hydrogen peroxide*

## TREATMENTS WHICH DID NOT EXTEND MUSHROOM SHELF LIFE

It is important that the treatments which had little or no effect on mushroom quality are recorded so that the work is not repeated unnecessarily by others. Special attention should also be paid to the effects of sodium metabisulphite: the experimental data here suggest that it offers no benefit at the concentration which has been applied in some mushroom processing companies.

### Antimicrobial compounds

#### Sodium metabisulphite

Sodium metabisulphite has been used extensively in the mushroom industry as a preservative and as a whitening agent. Due to allergic responses to sulphite in some asthmatics the industry is keen to replace it. The effects of sodium metabisulphite on mushroom keeping quality were monitored. This data was necessary as any potential replacement additive must be shown to be at least as effective as sodium metabisulphite.


▲ ***Fig. 10:*** *After 14 days at 4°C sodium metabisulphite treated mushrooms (right) looked similar to the control mushrooms (left)*

The standard method for assessing treatments was followed using a solution of 1 g/l sodium metabisulphite with a soaking period of 2 or 10 min. Mushrooms from flushes one, two and three, and from phase II and III compost were tested and the experiments were duplicated. The effect of the treatments on mushroom quality was unexpected. The shelf life (days for L value to drop to 76) of the sodium metabisulphite treated mushrooms was either the same or shorter than control mushrooms soaked in water, regardless of the mushroom flush or phase. Figure 10 shows the similarity in appearance between control and

sulphite treated mushrooms after 14 days chilled storage. Further investigations showed that the concentration of sulphur dioxide inside mushrooms 18 h after treatment was less than 5 mg/kg but the minimum concentration of sodium metabisulphite required to inhibit the growth of a *Pseudomonas* strain (isolated from fresh mushrooms) for more than 7 days in broth was found to be 2 g/l. The lowest sodium metabisulphite concentration tested, 0.1 g/l, was found to reduce the bacterial growth slightly but even this concentration is much greater than that found in the treated mushrooms. If sodium metabisulphite is to be used in mushroom processing then concentrations considerably higher than 1 g/l must be used. Under current legislation the maximum sulphur dioxide concentration permitted in processed mushrooms is 50 mg/kg. This concentration is unlikely to have a beneficial antimicrobial effect. The whitening activity of sodium metabisulphite on mushrooms was also disappointing. The mean Hunter "L" value for whole mushrooms dipped in a solution of 1 g/l sodium metabisulphite for 2 min only increased from 94.6 to 96.0 with a standard deviation of 1.6, and only increased for those dipped in 32 g/l from 95.8 to 96.9, with a standard deviation of 1.0.

These experimental data are important as they support the recommendation that sodium metabisulphite should not be used in the mushroom industry.

### A mixture of citric acid and hydrogen peroxide

Treatments with citric acid and hydrogen peroxide individually were very effective at extending mushroom shelf life. A mixture of the substances was also tested to see if it could extend shelf life yet further. Whole mushrooms were soaked in a mixed solution of 40 g/l citric acid and 5% (v/v) hydrogen peroxide. The standard test method was then followed. The effect of the mixed treatment was no better than the effect of treating mushrooms with solutions of either 40 g/l citric acid or 5% (v/v) hydrogen peroxide and so the use of this mixture is not recommended.

### Nisin

Nisin is a natural food preservative (a bacteriocin) which is effective against Gram positive bacteria. However, the common spoilage bacteria found on mushrooms (pseudomonads) are Gram negative. For nisin to have an antimicrobial effect on Gram negative bacteria, the bacterial wall must first be damaged by a substance such as EDTA. The effect of nisin on mushroom quality was tested using the standard method for assessing treatments. The treatment solutions were nisin (100 µg/ml), EDTA (10 g/l) and a mixture of nisin (100 µg/ml) and EDTA (10 g/l). The results showed that nisin, used alone or mixed with EDTA, had no beneficial effect on either whiteness or bacterial number.

### Diacetyl

Diacetyl is a volatile diketone with a strong buttery aroma. It has antibacterial properties and is particularly effective at low temperatures (Archer, 1994). The standard method for assessing treatments was followed using treatment solutions of 0.1, 1, 10, 50, 100, 250 and 500 mg/l diacetyl. There was no significant difference in colour between the diacetyl treated mushrooms and the control mushrooms which had been soaked in water alone.

## Antioxidant treatments

Several antioxidants were tested to see if they could extend mushroom shelf life. It was anticipated that they might inhibit the oxidation reaction involved in enzymatic browning. In the past, synthetic antioxidants have been used in some foods to retard lipid oxidation. However, some synthetic antioxidants have since been shown to have carcinogenic activity. Since the early 1980s there has been a trend to identify and develop natural antioxidants. Several are now widely used to inhibit lipid oxidation and so only natural antioxidants were screened for improving the shelf life of mushrooms.

### Vitamin E

Vitamin E (tocopherol) is a natural antioxidant which quenches free radicals thus making them inactive and harmless. It has been successfully used to increase oxidative stability and maintain good colour in beef (Brennan, 1997). The standard method for assessing treatments was followed using a solution of 2 g/l vitamin E. The treated mushrooms were slightly whiter than the control mushrooms on the seventh day of storage, but were less white than the control mushrooms on the tenth and fourteenth day of storage. The effect was not improved by doubling the vitamin E concentration. The lack of effectiveness might have been due to the poor solubility of vitamin E and so in an attempt to improve the contact of Vitamin E with the mushroom it was mixed in corn oil and emulsified in water using lecithin. However, mushrooms treated with this mixture were of no better colour than control mushrooms (soaked in water).

### Ascorbic acid (Vitamin C)

Ascorbic acid continues to gain importance as a versatile food additive as it improves the quality and shelf life of many food products. The effects of solutions of 20 and 40 g/l ascorbic acid on mushroom shelf life were tested using the standard method. The quality of mushrooms treated with the 20 g/l solution was very similar to that of the control mushrooms, but those treated with the 40 g/l solution were whiter and their shelf life (days to reach an L value of 76) was a day longer than that of the control mushrooms. Although ascorbic acid treatments were found to have some beneficial effects in extending the shelf life of sliced mushrooms they were less effective than treatments with citric acid and hydrogen peroxide. The effect of a mixture of citric acid and ascorbic acid was also assessed using the standard method, but its effect on shelf life was the same as that of citric acid alone. For these reasons trials with ascorbic acid were discontinued.

### Rosemary extracts

The beneficial effect of some herbs and spices on fat stability has been known for many years. Recently there has been considerable interest in

rosemary as it has been shown to be one of the most effective natural antioxidants available (Madsen and Bertelson, 1995). Rosemary extracts were supplied by two companies (Guinness Chemical, Portlaoise; Kalsec UK Ltd, Mildenhall Suffolk, UK) in water soluble formulations. Each formulation was tested at the concentration recommended by the supplier and also at twice this concentration, using the standard assessment method. All but one of the formulations were detrimental to mushroom whiteness, largely because the extracts were a brownish colour. The only formulation which had a beneficial effect was "Duralox" which is a mixture of rosemary extract, citric acid and ascorbic acid. The benefit from "Duralox" may have been due only to the citric and/or ascorbic acids.

## Packaging treatments

Consumers may find novel packaging systems more acceptable than food additives for extending the shelf life of sliced mushrooms. The aims of packaging currently used in the mushroom industry are to reduce desiccation and to reduce condensation onto the mushrooms, and overwrapping films are perforated to prevent anaerobic atmospheres in the packs. The economics of using novel packaging systems to extend the shelf life of mushrooms must be carefully considered as packaging can easily inflate production costs by too much.

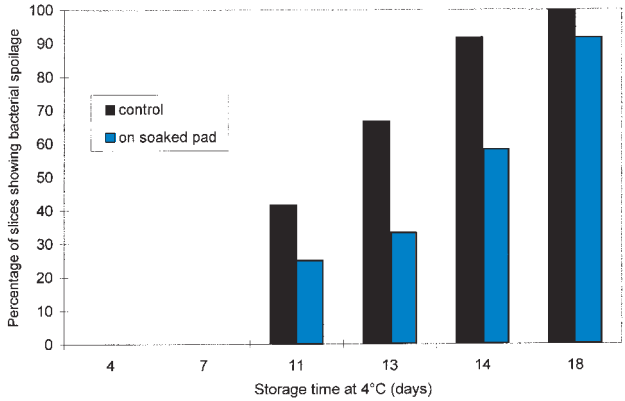
### Water control

The movement of water in mushroom packs can influence shelf life considerably. Unlike most fruit and vegetables, mushrooms have no barrier to water loss, so without packaging, mushrooms rapidly dehydrate. However, even with packaging, problems occur due to water movement. When packs are overwrapped with plastic film the atmosphere inside the pack becomes humid and condensation forms. Moisture on the mushroom surface encourages bacterial growth and shortens the shelf life. Thus, to extend shelf life it was hypothesised that packs should be wrapped, keeping a humid atmosphere around the mushrooms, but water should be prevented from condensing onto the mushrooms. The potential of using absorbent material,

▲ ***Fig. 11:*** *Mushrooms packed on absorbent pads*

with a "stay-dry" cover to separate absorbed water from the mushroom surface, was investigated.

The standard method for assessing treatments was followed except that the mushrooms were briefly rinsed instead of soaked and absorbent pads were placed under the mushrooms (Fig. 11). The mushrooms on the pads remained whiter than the control mushrooms but they became dehydrated: water was drawn out of the mushrooms into the absorbent material. To overcome this, water was added to the absorbent material before the mushrooms were packaged. This treatment reduced bacterial spoilage (Fig. 12) and thus extended shelf life, but the results varied considerably from batch to batch of mushrooms. To control water movement effectively, the absorbent material, the added water, and the water content of the mushroom must all be in the correct proportions. This is unrealistic because of the variation in mushroom water content. Other drawbacks are that the method is specific to small packs of mushrooms and the added packing components would be costly.




▲ ***Fig. 12:*** *Effect of soaked pad under mushrooms on bacterial spoilage of mushrooms stored at 4°C*

## Modified atmosphere packaging

In a pack of mushrooms a modified atmosphere is created by mushroom respiration, *i.e.* oxygen uptake and carbon dioxide evolution. The concentrations of oxygen and carbon dioxide within the pack are determined by initial gas composition, the rate of gas permeating through the packaging film, and the rate of respiration. To control these rates is difficult because they are affected by temperature, respiration rate varies considerably between mushroom batches, and film permeability varies due to uneven thickness and uneven sized micropores. The initial gas composition can be varied by flushing the mushroom packs with different mixtures of oxygen, carbon dioxide and nitrogen. Theoretically, to increase mushroom shelf life, the gas composition in a pack should have a low oxygen concentration, but never zero. Absence of oxygen within a mushroom pack results in anaerobic metabolism which produces off-flavours. It also increases the risk of production of botulin, a highly toxic compound which can be produced by *Clostridium botulinum* bacteria if the pack is kept above refrigeration temperature.

There has been considerable interest in modified atmosphere packaging and yet it has been largely unsuccessful for mushrooms. This is because mushrooms have a high respiration rate (500 mg/h $CO_2$ produced per kg mushrooms compared with, for example, 126 mg/h $CO_2$ produced per kg cauliflower) causing oxygen levels to rapidly deplete. A suitable packaging system might involve a triggered release of oxygen when the oxygen concentration in the pack reaches a critical level; however, technology for this is unavailable. What is available, is a range of packaging films with different permeabilities. Five film types were tested in this project.

Whole mushrooms were packed in bags and flushed with gas mixtures ($CO_2$:$O_2$:$N_2$ - 10:0:90, 15:5:80, 20:10:70, 20:0:80) or air, and stored at 4°C. The bags were made from four film types: low density polythene, polyester/polyethylene laminate, machine glazed polypropylene and a microperforated film allowing free passage of air. The gas composition in the bags and the colour and texture of the mushrooms were monitored over time. The results showed that none of the packaging regimes were effective in improving mushroom shelf life and many of the packs became anaerobic.

The fifth film tested was a new polyethylene film (25 micron metallocene film). Mushrooms were soaked in chilled water for 10 min, then sliced and packed. The packs were wrapped either with the standard perforated PVC film or with the metallocene film, non-perforated or with two or nine perforations. The mushrooms were stored at 4°C for 18 days and their colour, mass, and shear value were monitored. There was no significant difference (P= 0.01) in whiteness or shear value between the mushrooms wrapped with the different films. However, the metallocene film reduced water loss from the pack, even when it was perforated. Water lost from the mushrooms condensed on the underside of the film which was unsightly. In addition, the metallocene film had a poor sealing quality.

## SPICED MUSHROOM SNACKS

### Introduction

The main emphasis of the project reported here was to extend the shelf life of *fresh* sliced mushrooms. However, extension in shelf life was also investigated from another angle: to develop a new mushroom product with a long shelf life that could be exported to distant destinations. The shelf life of foods can be increased by making them unfavourable for bacterial growth by removing water and adding inhibitors such as salt (NaCl). Loss of water means loss of weight and volume and consequently, dehydrated mushrooms are cheaper to transport than fresh mushrooms. Many methods of water removal are very expensive due to their high energy demands; osmotic dehydration, however, is a relatively cheap technique. It is defined as "water removal by immersion of a water-containing cellular solid (*e.g.* mushroom) in a concentrated aqueous solution (*e.g.* brine)". When a mushroom is soaked in a concentrated salt solution water moves from the mushroom to the salt solution, thus dehydrating the mushroom. When the salt concentration inside and outside the mushroom cells is equal there is no further net water movement. Osmotic dehydration has the potential, not only for removing water, but also for introducing salt and flavour compounds to the mushroom.

However, it must be combined with an additional drying procedure, such as freeze-drying, to ensure crunchy dry snacks.

The savoury snack market was considered to be a suitable niche for a mushroom product with a long shelf life. Most of the savoury snacks already in the market place are low in sugar but high in salt and fats. The new mushroom snack described here has the advantage that it is low in fats.

## Product development

Development of the new snack product involved optimising the mushroom slice thickness, the salt concentration of the soaking solution and the duration of the osmotic dehydration process. A slice width of 3 mm, and a 10-20 min soak in a 20% (w/v) salt solution were selected.

The distinctive mushroom aroma and taste were carried through to the snack product and were pleasantly stronger than in fresh mushrooms but to make the flavour of the mushroom snacks more interesting, spicy flavours were added. Several flavour compounds were tested by adding them to the soaking solution, but the addition of a mixture of herbs and spices was preferred by a taste panel.

Some batches of spiced mushroom snacks tasted too salty. This is probably due to differing water contents of different batches of mushrooms. As water content varies, the solute concentration in the mushroom may also vary. The rate of osmosis and the amount of water that can be removed by osmotic dehydration depends on the original solute concentration. This, in turn, affects the amount of salt taken up by the mushrooms. To obtain mushroom snacks with consistently acceptable levels of salt the osmotic dehydration process would have to be adjusted for each batch according to their solute concentration or water content. A rapid test on each batch of mushrooms could be used to determine the appropriate soak time and/or salt concentration of the soaking solution. It may also be possible to use automatic feedback control to adjust the soak time according to the initial rate of dehydration.

The aim of the osmotic dehydration, was not only to obtain the desirable texture and flavours, but also to minimise the duration of the freeze-drying

▲ ***Fig. 13:*** *The research group tasting mushroom snacks*

step. Alternatives to freeze-drying could also be considered. Pilot-scale vacuum microwave drying is currently being investigated elsewhere as a secondary process after osmotic treatment of fruit. The process takes less than an hour and may therefore prove to be more suitable and economical than freeze-drying. If this is found to be true, then further tests to overcome variation of salt uptake and to determine product shelf life should be carried out prior to industrial production of spiced mushroom snacks. A more detailed description of this sub-project has been published recently (Brennan and Salmier, 1998).

## CONCLUSIONS

- The aim of the project, to extend the shelf life of sliced fresh mushrooms by 50%, was achieved.
- Mushrooms soaked whole in a solution of 40 g/l citric acid for 10 min prior to slicing and packing had a 50% longer shelf life than mushrooms soaked in water.
- This treatment had no undesirable effect on cooked mushroom flavour and is permitted under current regulations.
- Treatments with EDTA (40 g/l) or hydrogen peroxide (5%) were also effective in extending mushroom shelf life.
- Treatment with sodium metabisulphite (1 g/l) was ineffective in prolonging shelf life and only whitened mushrooms slightly.

- Effectiveness of the treatments varied from batch to batch of mushrooms.
- The least tough batches of mushrooms responded best to the treatments.
- There was no difference in whiteness for Silvan strain mushrooms from flushes 1, 2 or 3 and from phase II or III compost.
- Treatments with diacetyl, nisin, vitamins C and E and rosemary extracts had no beneficial effect on mushroom shelf life.
- Modified atmosphere packaging is unsuitable for mushrooms because they have a high respiration rate.
- Moist absorbent material inserted underneath mushroom slices improved mushroom shelf life slightly but the effectiveness varied from batch to batch of mushrooms.
- The development of a novel product, spiced mushroom snacks, with an extended shelf life, has been described.

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