Novel practice to produce safe and healthy dry fish using irradiated chitosan coating

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Abstract

In Bangladesh, dry fishes are mostly produced by drying in an open environment under sunlight, and so the producers are forced to use insecticides and fungicides to prevent microbial and blowfly infestation. That is why dry fishes are often contaminated with residual insecticides and fungicides, which cause a severe threat to human health. In this experiment, gamma radiation treated chitosan solution was used while drying the fishes, and the effect was evaluated. Experimental results suggested that chitosan coating did not hinder the drying process but improved the quality of the produces. Chitosan coating was found as a repellant of blowflies as well. Besides, it also prevented microbial contamination. The nutritional value of the produced dry fishes was examined and found very satisfactory. Overall results suggested that the application of irradiated chitosan can be a very suitable and cost-effective way to produce safe and healthy dry fishes.

1. Introduction

In the sense of food concern, food security exists when all people from all classes have the basic and safe food they need. In Bangladesh, malnutrition remains all around, especially in the case of protein source food. In this regards, the high protein-containing dry fish can be a great source to meet the demand because the dry fishes are the predominant food bringing vital protein to people in rural areas of least developed countries (Graikoski, 1973). As Bangladesh is the sixth-largest fish producing county in aquaculture (Momtaz and Shameem, 2015), there is a huge opportunity for preserved fish in the country. But as a perishable nature and due to costly management system as fresh produce, in a developing country like Bangladesh, the drying method is considered as the least expensive method of fish preservation (Balachandran, 2001).

The drying method is the oldest food preservation technique and available in many chemical and processing industries. The eradication of moisture halts the rapid growth of microorganisms, causing decay and lessen much moisture mediated unexpected reactions (Kilic, 2009). About 15% of fishes are cured for mass people consumption at the scarcity of fresh fishes in Bangladesh (Khan and Khan, 2001; Khan and Khan 2002). It is also a very favorite food item among Bangladesh people and has a good market demand besides fish and other seafood products. Moreover, in the case of some marine fish species, e.g. loitta (Bombay duck), knife fish etc., people do not like to consume...
fresh fish, but they like to eat dry fish of these species (Siddique and Akhtar, 2011). Moreover, dry fish has a storage life of several years and is a great source of protein, essential fatty acids, vitamins and a lot of minerals (Siddique and Akhtar, 2011). So, it is consumed all over the world for its nutritional value, taste, and aroma.

During the rainy season, when humidity levels are high, and the sunlight is very low, sufficient drying cannot be achieved using traditional methods. Moreover, stored dried fish re-absorbs moisture in such conditions and becomes susceptible to bacteria, fungal or insect attack (Azam, 2002). In the Indonesian code of practice, for fresh and cured fish (Walker, 1984), it was stated that when the relative humidity was greater than 75%, rehydration of dried fish happened if the product was not wrapped with polythene or another appropriate packaging. Wood (1981) stated the same opinion that in an atmosphere with a humidity greater than 60%, the dry fish tended to pick up moisture, with a consequent risk of spoilage (Khan and Khan, 2001). For these reasons, dry fish farmers often use insecticides for protecting raw fish from insect attack, which is a serious health concern (Samad et al., 2009). Besides, blowfly infestation in sun-drying fish is a major economic problem in many developing countries of Asia, Africa and the Pacific (Wall et al., 2001). Under warm and humid conditions, sun-drying fish can rapidly become infested by blowfly larvae (Kordyl, 1976). The infestation begins when adult females are attracted to the fish and lay eggs, particularly in the mouth and gills. The larvae feed initially in the body cavity, gills and eye sockets (Walker and Wood, 1986). On maturity, the larvae usually leave the fish and burrow into the soil beneath to pupate. The amount of damage caused by blowfly larvae depends largely on the speed of drying, the size of the fish, and whether the fish is salted or not (Walker and Wood, 1986). However, in extreme cases, larvae destroy all of the soft tissues, leaving only the skin, scales and bones (Walker and Donegan, 1984; Walker and Wood, 1986).

Fishers use a mixture of organochlorine insecticides to protect dry fish from infestation (Bhuiyan et al., 2008). Some analyses in Bangladesh showed alarming pollutants in fish like DDT and heptachlor (Bhuiyan et al., 2009; Chowdhury et al., 2010). In Kuakata (a fish processing zone in Bangladesh), a high level of DDT powder (locally known as white powder) is used, although Bangladesh banned the 'dirty dozen' in 1997 (Bhuiyan et al., 2009; Chowdhury et al., 2010). DDT is classified as "moderately toxic" by the US National Toxicological Program and "moderately hazardous" by WHO and a B2 probable human carcinogen (Bhuiyan et al., 2009; World Health Organization, 2010).

A natural bio-polymer, chitosan, can act as a chelating agent that chelate some metal ions needed by a bacterial enzyme belonging to cationized amines (Goy et al., 2009). It is known that cation – NH3+ can interrupt metabolism by interaction with other negative ions present at the bacterial cell wall, which causes internal osmotic imbalances and inhibits the growth of microorganisms (Hadwiger et al., 1986; Goy et al., 2009). Furthermore, some funguses are capable of producing carcinogenic ochratoxin A and aflatoxin, which may be inhibited by chitosan thorough chitinase activity in host tissues (Goy et al., 2009; Sirmats, 2009; Matan, 2012). Besides, different treatments can further boost the effectiveness of chitosan. Gamma radiation leads to chain scission of chitosan molecules and produces oligomers with even better antimicrobial activity (Ibrahim et al., 2014). In this experiment, 40 kGy gamma radiation dose was used to modify chitosan solution for better activity, optimized in our earlier experiments (Ibrahim et al., 2014).

Our present study evaluated the dry fish (Mola fish) preparation by dipping the fish in formulated chitosan solution and then allowed drying under sunlight. We have also observed the anti-microbiological effect of formulated chitosan as well as the efficacy of blowfly eradication.

2. Material and methods

2.1 Materials

Fresh Mola fish (Amblypharyngodon mola) were bought from the local market of Savar, Dhaka, Bangladesh. Fish were taken into a hermetically sealed container and brought into the laboratory for further experiment. Chitosan was extracted from the prawn shell in the Institute of Radiation of Polymer Technology (IRPT), Bangladesh Atomic Energy Commission laboratory by the standard chemical procedure (Rashid et al., 2012). The prepared chitosan was food grade, and the degree of deacetylation was 80-85%. All other chemicals were lab grade and purchased from a local chemical supplier.

2.2 Preparation of irradiated chitosan

Chitosan solution (2% w/v) was prepared using 2% acetic acid. The prepared solution was then irradiated by Co-60 gamma irradiator at 40 kGy with a dose rate of 6.93 kGy/hour.

2.3 Dry fish preparation

Mola fishes were washed with distilled water and...
then treated with different concentrations of chitosan solution (2000, 3000, 4000 and 5000 ppm). Both treated and untreated (control) fishes were divided into two groups and were kept for room temperature drying (indoor) and sunlight drying (outdoor) in the IRPT campus, Savar, Dhaka, Bangladesh. Samples kept under sunlight took three days for drying, whereas indoor samples took five days. It was reported that fish samples dipped in 2% chitosan solution (20,000 ppm) was safe for human consumption (Fan et al., 2009). However, in our experiment, we capped our treatment concentration at 5000 ppm to make sure the treatment is safer.

2.4 Microbiological analysis

Microbiological analysis was conducted by means of total bacterial count and total fungal count. 1 g of dry fish sample was ground by a sterilized grinder and vortexed in distilled water to make 10 mL dry fish powder suspension. This suspension was further used to estimate the microbiological load. Plate count agar media (Oxoid, UK) was used for the total bacterial count, and potato dextrose agar media (Oxoid, UK) was used for the fungal count. The pour plate technique was used for both of the tests.

2.5 Biochemical analysis

The quality of samples was investigated by estimating protein, fat and moisture content according to the standard procedure of the Association of Official Analytical Chemists (AOAC).

2.6 Blowfly infestation study

This study is done by observing the number of blowflies attracted to the samples. The blowfly counting was started after three hours of chitosan treatment. Each day, three readings were taken at 2 hours of intervals. The total weight of the samples per experimental group was 500 g, and the fishes were kept horizontally aligned.

2.7 Statistical analysis

Statistical analysis was conducted by SPSS software by means of standard deviation and one-way ANOVA.

3. Results and discussion

3.1 Microbiology of dried fish

Microbial activity is the main factor limiting the shelf life of fresh fish (Olafsdottir et al., 1997). In this experiment, the chitosan solution was used to inhibit microbial growth in the fish. Chitosan solutions were found to have a significant effect on controlling microbial growth at both room temperature and sunlight drying conditions. Bacterial counts were measured in 5000, 4000 3000 and 2000 ppm chitosan treated samples and untreated sample as 4.0, 4.63, 5.11, 4.98 and 5.33 Log_{10} CFU/g, respectively (Figure 1) in sunlight drying condition. These results suggested about 13 times higher bacterial growth in the untreated sample compared with the 5000 ppm chitosan treated sample. Similar results were found for the samples dried at room temperature (Figure 1); all chitosan treated samples got better results than untreated samples. The best outcome (4.47 Log_{10}CFU/g) was achieved for 5000 ppm irradiated chitosan treatment. It was reported that cationized amines of chitosan chelated negatively charged potassium ions and other proteinaceous constituents of the microbial cell wall, making them locally detached from the cell membrane. This chelation also enabled the passing through murein and plasma membrane. Thus, chitosan bounds with microbial DNA and modulated transcriptional and translational mechanism (Goy et al., 2009; Alishahi, 2012). Moreover, chitosan acted as a water chelating agent in dry fish surface and microbial cell wall, which led to the blocking of active enzyme groups and toxin biosynthesis (Alishahi, 2012). All of these mechanisms eventually led to microbial growth retardation on the dry fish surface.

According to Prasad et al. (1994), salt-treated dry fishes contained Valamugil speigleri pathogen of 7.38 Log_{10} CFU/g, which is about double of total bacterial count found for chitosan treated dry Mola fish. According to Jay et al. (2008), the minimal reported growth restriction temperature for foodborne microbial species was -5°C, whereas we found significant bacterial growth retardation at room temperature due to chitosan treatment.

Auspicious results were also obtained in the case of fungal inhibition (Figure 2). Fungal growth was found about nil in the case of 3000 ppm and higher doses of chitosan treatment under sunlight drying, whereas 5000 chitosan solution led to nil fungal growth for sample dried at room temperature. On the other hand, untreated...
干鱼样品的霉菌数为3.47CFU/g和4.24CFU/g，分别用于日光干燥和室温干燥。Kiliç（2009）和Patterson和Ranjitha（2009）发现冷干（20°C）虹鳟鱼鱼含有2.5-3.4Log_{10} CFU/g，和商业风干样品含有1.3Log_{10} CFU/g总菌数。因此，实验结果表明4000ppm的壳聚糖溶液保持了干鱼的霉菌数，但霉菌数保持了低水平，因为壳聚糖溶液不显著影响干鱼的霉菌数。Zivanovic et al.（2004）也发现相似结果；低浓度的醋酸在阳光下干燥的细菌数和霉菌数为4.20CFU/g。Ojagh et al.（2010）证实壳聚糖涂层可防止霉菌和真菌对鱼类的攻击。Flowra et al.（2012）研究了商业干鱼的5种干鱼。它反映了44.08%到65.65%的蛋白质含量，对于商业销售的干鱼来说。因此，在未经处理的样品中，没有发生风味、颜色和维生素的降解，而且任何低于此值的值可能表明由于鱼的蛋白质和脂肪等营养成分的转化导致的肉质降解，以及可能的风味、颜色和维生素的降解。（Augustini and Sedjati，2007）。

因此，在未经处理的样品中，风味、颜色和维生素的降解发生，而且任何低于此值的值可能表明由于鱼的蛋白质和脂肪等营养成分的转化导致的肉质降解，以及可能的风味、颜色和维生素的降解。（Augustini and Sedjati，2007）。

3.2 Biochemical analysis

生物化学参数如蛋白质、脂肪和水分含量的干鱼样品被估算并表示在表1中。没有显著性差异被发现于商业干鱼的营养值的处理样品和未经处理样品之间。然而，总的来说，未干鱼样品显示较低的水分含量和更高的蛋白质含量，而室温干燥样品的脂肪含量较高。

| Sample       | Moisture (%) | Protein (%) | Fat (%) |
|--------------|--------------|-------------|---------|
|              | Sun drying   | Room temperature drying | Sun drying | Room temperature drying | Sun drying | Room temperature drying |
| Control (Untreated) | 7.15          | 10.46        | 64.12    | 62.60        | 21.40      | 19.90                  |
| 2000 ppm     | 8.15          | 12.73        | 62.60    | 59.50        | 25.20      | 22.30                  |
| 3000 ppm     | 8.32          | 13.20        | 62.47    | 58.20        | 25.50      | 23.60                  |
| 4000 ppm     | 8.28          | 13.55        | 61.42    | 58.70        | 25.40      | 23.80                  |
| 5000 ppm     | 8.68          | 14.89        | 61.72    | 56.80        | 25.60      | 23.40                  |

图2. 干鱼样品的霉菌数。不同样本的差异有显著性差异（P<0.05）。

3.3 Pest (Lucilia cuprina) infestation studies

这项研究的目的是观测林福拉和孟加拉国的商业干鱼的林福拉 Crushers（Patterson和Ranjitha，2009）。

在我们的实验中，壳聚糖涂层处理的样品的蛋白质含量从56.8%到62.6%不等，这表明了其商业价值和营养接受度。

- 未经处理的样品的蛋白质含量为7.15%至14.89%。
- 4000ppm的壳聚糖处理样品的蛋白质含量为7.15%至14.89%。
- 5000ppm的壳聚糖处理样品的蛋白质含量为7.15%至14.89%。

因此，在未经处理的样品中，蛋白质、脂肪和其它营养成分被微生物生物质所吸收，而壳聚糖涂层减少了蛋白质含量，因为它对微生物有抗微生物作用。Alishahi和Aider（2012）和Ojagh et al.（2010）证实了壳聚糖涂层可以防止细菌和真菌的攻击。

因此，实验结果表明4000ppm的壳聚糖溶液保持了干鱼的霉菌数，但霉菌数保持了低水平，因为壳聚糖溶液不显著影响干鱼的霉菌数。Zivanovic et al.（2004）也发现相似结果；低浓度的醋酸在阳光下干燥的细菌数和霉菌数为4.20CFU/g。Ojagh et al.（2010）证实壳聚糖涂层可以防止细菌和真菌的攻击。Flowra et al.（2012）研究了商业干鱼的5种干鱼。它反映了44.08%到65.65%的蛋白质含量，对于商业销售的干鱼来说。因此，在未经处理的样品中，风味、颜色和维生素的降解发生，而且任何低于此值的值可能表明由于鱼的蛋白质和脂肪等营养成分的转化导致的肉质降解，以及可能的风味、颜色和维生素的降解。（Augustini and Sedjati，2007）。

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of blowfly in the fish samples (500 g). It was found that the blowflies attracted by the fish samples were reduced with the sunlight intensity. The blowfly infestation was also reduced with the degree of drying of the fish samples. On the other hand, the chitosan treated sample showed significant low blowfly infestation compared to the untreated control samples at both room temperature (simulation of cloudy day drying) and sunlight drying. In the case of sun-dried untreated control samples, the maximum fly infestation was found 19 at Day 2, 10.00 am. Whereas chitosan treated sample showed only two fly infestation at the same time for 5000 ppm treatment. Similar results were also found for the room temperature dried samples. The maximum fly infestation was 30 for the untreated control on Day 2, 2.00 pm. 3000 ppm chitosan treated sample was affected by only eight flies in the same period (Table 2, Figure 3). It was also found that the blowflies laid significantly less egg on the chitosan treated samples.

A second trial was conducted to evaluate whether the pest repellent effect was due to the chitosan coating or the odor of acetic acid. The trial confirmed that blowflies were attracted to not only untreated fish but also to acetic acid-treated fish samples (Table 3). Becher et al. (2010) also reported that the flies were strongly attracted the odor of acetic acid as the flies had an odor-mediated behavior to find out their feed.

![Figure 3. Blowfly infestation on dry fish samples; A (Control), B (5000 ppm chitosan treated).](image)

It was also found that the blowflies ate the fish flesh that led to hallow structure formation in the fish sample (Figure 4). The experiment showed that blowfly easily penetrated, laid eggs in the untreated fish samples. The hatched larvae infected the fish samples and ate the soft flesh of the fish.  

| Table 2. Blowfly infestation on different dry fish samples |
|----------------------------------------------------------|
| **Blowfly count**                                        |
| | Day 01 | Day 02 | Day 03 |
| | 10.00 am | 12.00 pm | 2.00 pm | 10.00 am | 12.00 pm | 2.00 pm | 10.00 am | 12.00 pm | 2.00 pm |
| Sun-dried sample | | | | | | | | | |
| Control | 4 | 8 | 6 | 19 | 14 | 12 | 7 | 0 | 0 |
| 2000 ppm | 2 | 1 | 2 | 9 | 5 | 2 | 1 | 0 | 0 |
| 3000 ppm | 2 | 0 | 1 | 8 | 3 | 7 | 2 | 0 | 0 |
| 4000 ppm | 2 | 1 | 1 | 5 | 4 | 1 | 1 | 0 | 0 |
| 5000 ppm | 2 | 1 | 0 | 2 | 2 | 0 | 0 | 0 | 0 |
| Room temperature dried sample | | | | | | | | | |
| Control | 3 | 6 | 10 | 26 | 31 | 30 | 29 | 21 | 20 |
| 2000 ppm | 2 | 2 | 4 | 7 | 8 | 10 | 11 | 8 | 5 |
| 3000 ppm | 2 | 3 | 2 | 5 | 5 | 8 | 4 | 4 | 2 |
| 4000 ppm | 1 | 2 | 3 | 5 | 4 | 6 | 3 | 3 | 2 |
| 5000 ppm | 1 | 1 | 2 | 4 | 3 | 7 | 3 | 4 | 2 |

| Table 3. Blowflies count of control, solvent treated (acetic acid) and 3000 ppm chitosan treated fish samples during drying under sunlight |
|----------------------------------------------------------------------------------------------------------------------------------|
| **Day** | **Time** | **Number of blowflies on untreated dry fishes** | **Number of blowflies on acetic acid treated dry fishes** | **Number of blowflies on chitosan treated dry fishes** |
| | | | | |
| 1<sup>st</sup> day | After one hour | 1 | 1 | 0 |
| | After two hours | 1 | 1 | 0 |
| | After three hours | 1 | 1 | 1 |
| | After four hours | 2 | 2 | 0 |
| | After five hours | 2 | 2 | 1 |
| 2<sup>nd</sup> day | After one hour | 13 | 10 | 1 |
| | After two hours | 15 | 11 | 0 |
| | After three hours | 13 | 12 | 2 |
| | After four hours | 9 | 7 | 2 |
| | After five hours | 6 | 6 | 1 |
| 3<sup>rd</sup> day | After one hour | 14 | 12 | 1 |
| | After two hours | 15 | 16 | 1 |
| | After three hours | 9 | 11 | 1 |
| | After four hours | 10 | 9 | 0 |
| | After five hours | 11 | 9 | 1 |
tissues. In comparison, no hallow structure was found for the chitosan treated fish samples. Some researchers claimed that, nevertheless, using salt in dry fish processing, blowfly could easily pierce all of the soft tissue except only the skin, scales and bones (Walker and Donegan, 1984; Walker and Wood, 1986). However, our current investigation showed that no damage occurred to the soft tissues of chitosan treated dried fish.

It can be assumed that the chitosan coating acted as a barrier for gas permeability and thus led to decreased smell emission from the fish samples. Moreover, it was found that chitosan solution had significant antibacterial and antifungal activity from the antimicrobial studies. So, the chitosan treated fishes had reduced flesh putrefaction and thus emitted less smell to attract blowflies. The combined effect of these two mechanisms ultimately led to the blowfly repellant characteristics of chitosan treatment.

4. Conclusion

The major challenges of producing dry fish are the blowfly attack and microbial degradation of the fish, especially when sunlight is absent for several days. In this experiment, we found the irradiated chitosan had a significant role in overcoming the challenges. Chitosan treatment was found to control both the blowfly infestation and microbial attack. Among the treatments 5000 ppm, chitosan solution showed the best results. However, the results for the 3000 ppm chitosan solution were also quite promising. Chitosan treatment did not show any effect on the drying time of the fish samples or the nutritional values of the dry fish. So, irradiated chitosan treatment before fish drying can be a very effective technique to produce healthy dry fishes without losing their quality and quantity. This practice will also discourage dry fish producers from using harmful chemicals and pesticides as they can now get rid of blowfly and microbial attack using human health-friendly chitosan treatment. Thus the research can play a critical role to ensure food safety and security worldwide.

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