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TECHNICAL NOTES

Microbiological Assessment of Locally Dried Fish in Oman: Technical Note

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التقييم الميكروبيلوجي للأسماك المجففة محليًا في عمان: ملاحظة فنية

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Abstract. The presence of microorganisms is one of the major factors affecting the quality of dried fish. In this project, a number of analytical tests were used to verify the presence of biochemical contamination in dried fish, as well as to propose an engineering solution to reduce the incidence of these contaminations. Two types of fish samples (anchovy and shark) were collected from two local markets (Buraimi and Seeb). The analyzed parameters included Total Fungal Count (TFC), Total Viable Count (TVC) and enumeration of Escherichia coli (E. coli), Staphylococcus aureus and Coliforms species in dried fish. The results indicated that the anchovy collected from Seeb market had the highest TVC and TFC (TVC= 5.59×10^5 cfu/ml, TFC= 3.60×10^4 cfu/ml) compared to all other samples. It could be recommended to dry fish under more hygienic conditions using solar drier instead of direct drying on beach sands.

Keywords: Solar drying, fish drying, fish contamination, dried fish, microbial assessment

المستخلص: يعتبر وجود الكائنات الحية الدقيقة أحد أهم العوامل الرئيسية التي تؤثر على جودة الأسماك المجففة، وفي هذه الدراسة تم استخدام عدد من الأختبارات التحليلية للتحقق من وجود الملوثات البيلوجية في الأسماك المجففة محلياً، وكذلك تم اقتراح حل هندسي يعمل على تقليل وجود هذه الملوثات، ومن أجل ذلك فقد تم جمع عينات لنوعين من الأسماك وهما الأنشوجة والقرش (تسمى محليا القاشع و العوال) من سوقين محليين وهما البريمي والسيب، وقد تم تحليل وجود العوامل التالية: العدد الكلي للفطريات (TFC) والعدد الكلي الحيوي (TVC) وتعداد بكتيريا *E-coli* و بكتيريا Staphylococcus aureus وبكتيريا الكوليفورم في الأسماك المجففة. وقد أظهرت النتائج أن العدد الكلي للفطريات (5.59×10^5 cfu/ml) والعدد الكلي الحيوي (3.60×10^4 cfu/ml) في الأنشوجة التي تم جمعها من سوق السيب كان أكثر منها في العينات الأخرى. وبناء على وجود هذه الملوثات في الأسماك المجففة فإننا نوصي بتجفيف الأسماك في ظروف صحية أفضل، ونشجع على استخدام المجففات الشمسية بدلا من التجفيف المباشر على رمال الشاطىء.

الكلمات المفتاحية: التجفيف الشمسي ، تجفيف الأسماك ، تلوث الأسماك ، الأسماك المجففة ، التقييم الميكروبيلوجي.

Introduction

Fish is the main foodstuff around many countries in the world because of its high protein content and nutritional value. However, in the hot climates, fish perishes quickly and it is common to increase their shelf life using different techniques such as drying, smoking and salting (Sultana et al., 2010). In the Gulf region, Oman is the largest fish producer (FAO, 2019) and it has more than 150 kinds of fish and crustaceans (Belwal et al., 2015). Most of the fish in Oman are sold fresh (93%) and the remaining 7% is sold after being dried, salted or smoked (FAO, 2019). In 2018, the total fish production of Oman was about 553 thousand tons which was 59% more than the production in the previous year (MAF, 2018). In the same year, fish export constituted 44% of the total production (about 245 thousand tons).

The traditional drying process of fish causes the loss of 30-40% of dried fish due to eating by dogs, birds, cats, and rats, especially during winter when drying takes about a week (Sablani et al., 2002). Indeed, this factor causes a reduction on the revenue of dried fish, which also suffers from the contamination by sand particles. It was reported that sand contamination causes high concentration of ash in the dried fish (Al Ghabshi et al., 2012). In addition, sun-dried fish may become unhealthy

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when it is attached by insects and larva. It was found that some fishermen apply insecticides to avoid this kind of attacks (Bala & Mondol, 2001).

The presence of microorganisms affects the quality of dried fish. Some studies showed that bacteria, molds, and yeasts are potential microorganisms that usually contaminate dried fish (Akwuobu et al., 2019). Therefore, it is important to determinate the microbiological quality of dried fish. The aim of this study was to verify the presence of microbiological contamination in dried fish collected from selected local markets; and to propose an engineering solution to reduce these contaminations.

Methodology

Two common types of traditionally dried fish in Oman were collected namely; anchovy and shark (locally called Owal) from two different places; Buraimi and Seeb fish markets. The anchovy samples were collected from packaged and unpackaged conditions from the two places. About 500 g was purchased from each place. They were placed in zipped bags and transported to the Food Microbiology laboratory at the College of Agricultural and Marine Sciences, SQU for analysis.

Media Preparation

All media were prepared according to Al Bulushi (2017) and each test was prepared using the media as mentioned in Table 1. After preparing the media for each test, it was mixed with distilled water. Then, each mixture was poured in a bottle and autoclaved at 121°C for 2-2.5 h. After autoclaving, 15 ml of the agar media was placed in Petri dishes and cooled at room temperature to solidify the media (Al Bulushi, 2017).

Total Fungal Count and Total Viable Count

From each sample, 25 g was placed in a sterile stomacher bag, and 225 ml of maximum recovery diluent was added in the stomacher bag (Interscience/France) to achieve 1/10 (10^{-1}) dilution. Then, 10^{-2} and 10^{-3} dilutions were prepared. For plating, 0.1 ml (100 µl) was transferred from each tube of the 10^{-1}, 10^{-2}, and 10^{-3} dilution mixtures to duplicate plates of the Potato Dextrose Agar (PDA) plate aseptically. Then, the mixture was spread using alcohol flamed spreading from the highest dilution to the lowest dilution (i.e. 10^{-3} to 10^{-1}). All plates were incubated aerobically at an ambient temperature of nearly 23°C for 3-5 days. After that, the colonies on plates were counted and reported (cfu/g) using Equation 1.

For Total Viable Count, all procedures were the same as Total Fungal Count except the differences with the type of agar used (SPCA), temperature (35°C) and period of incubation of the plates (48 h).

Enumeration of Bacteria

The samples were stored in a freezer at -40°C until used for the microbial analysis. Then, the samples were analyzed for 3 types of bacteria; *E. coli, staphylococcus aureus* and *coliforms* as total colony-forming units (cfu/ml) using different agar media, as explained below.

Enumeration of *E. coli, Staphylococcus aureus* and Coliforms

For *E. coli*, about 50 g of fish sample was taken, cut into small pieces and mixed together. Then, a stomacher bag was placed in a beaker and only 25 g of the mixed pieces were placed in the bag. After that, 225 ml of diluent was added to the stomacher bag and blended for 1 min to achieve a homogenized mixture at 10^{-1} dilution. Then, dilutions of 10^{-2} and 10^{-3} were prepared.

For plating, 0.1 ml from each tube of the 10^{-1}, 10^{-2}, and 10^{-3} diluents were transferred to duplicate plates of Tryptone Bile X-Glucuronide Agar (TBXA) plate aseptically. Then, the mixture was spread using alcohol flamed spreading from the highest dilution to the lowest dilution (i.e. 10^{-3} to 10^{-1}). Finally, incubation was done for all plates aerobically at 35°C for 24 h and only blue or green colonies were counted and reported (cfu/g) using Equation 1.

For *staphylococcus aureus*, the diluents were prepared as explained earlier for the enumeration of *E. coli* in dried fish. For plating, molten Baird-Parker agar were used instead of molten TBX Agar. Finally, black colonies were counted and reported (cfu/g) using Equation 1.

For coliform species, the diluents were prepared as explained earlier for the enumeration of *E. coli* in dried fish. For plating, 1 ml from each diluent was transferred to sterile petri dishes. For each dilution, two plates were used. Over a period of 15 min, approximately 12 ml of molten VRB Agar at 44-47°C was added. The medium with the inoculum was carefully mixed by horizontal movements (pour plate method) and the medium was allowed to cool down. Finally, the dishes were incubated for 24±2 h at 35°C and only pink colonies were counted and reported (cfu/g) using Equation 1.

Moisture Content and Water Activity

The moisture content was determined by oven-drying the samples at 105°C for 24 h (Ullah et al. 2016). The water activity (a_w, unit less) of the sample was determined using a water activity meter. The correlation of moisture content and water activity with the analyzed microbial counts was studied using STATA 13.0 (StataCorp, USA) at $\alpha = 0.05$.

$$cfu = \sum_{n=1} \text{number of colonies on the plate} \times \frac{1}{\text{dilution factor X volume taken}} \quad (1)$$

Table 1. The media used for each biological test.

Tests	Media	Manufacturer/country
Total Fungal Count	Potato Dextrose Agar (PDA)	Sigma-Aldrich/USA
Total Viable Count	Standard Plate Count Agar (SPCA)	Sigma-Aldrich/USA
The diluent	Maximum Recovery Diluent	Sigma-Aldrich/USA
Enumeration of E. coli	Tryptone Bile X-Glucuronide Agar (TBXA)	Sigma-Aldrich/USA
Enumeration of Staphylococcus aureus	Baird Parker Agar (BPA)	Sigma-Aldrich/USA
Enumeration of Coliforms species	Violet Red Bile Agar (VRBA)	Sigma-Aldrich/USA

Results and Discussion

Microbial Contamination

The anchovy collected from Seeb market had the highest TVC and TFC (5.59×10^5, 3.60×10^4 cfu/ml, respectively) as compared to all other samples (Table 1). In addition, the presence of 3 species of bacteria; *E. coli, staphylococcus aureus*, and coliforms was detected in anchovy samples more than in shark samples as collected from the two markets. Previous studies reported that anchovy had more contamination than the allowable levels (Aliya et al., 2018; Kumar et al., 2017). It was observed that the moisture content and water activity of the shark from Buraimi market were the highest among all samples (30.37% and 0.74, respectively). In the case of dried fish storage, Bala and Mondol (2001) stated that microbial growth was inhibited when the moisture content was reduced to 25% and particularly mold growth could be avoided when the moisture content was not more than 15%. However, this was not the case in this study, as reported in Table 2, which could be related to the types of contaminations present in the samples used in this study. Although, the moisture content of anchovy was lower than 15%, large values of TVC and TFC were observed.

Table 2. Results obtained from different tests.

Fish Type (Market)	Xm (%)	aw	TVC=APC (cfu/ml)	TFC (cfu/ml)	E. coli (cfu/ml)	S.aureus (cfu/ml)	Coliforms (cfu/ml)
Shark (Seeb)	23.66	0.64	1.05×10^3	2.50×10^2	0	6.96×10^3	0
Shark (Buraimi)	30.37	0.74	1.05×10^4	2.00×10^3	0	1.33×10^4	0
Anchovy (Seeb)	14.54	0.69	5.59×10^5	3.60×10^4	4.77×10^2	1.64×10^6	1.78×10^3
Anchovy (Buraimi)	14.15	0.65	1.29×10^5	3.70×10^3	6.00×10^2	2.27×10^5	2.82×10^2

The detected microbes in the analyzed samples could have come from several sources, namely, unhygienic handling of fish during drying and storage, and birds and animals' droppings. In addition, long time open-sun drying under high humidity environments enhances the growth of microorganisms (Sablani et al., 2002). In general, major sources of contamination include polluted coastal water and soil, dust and unhealthy treatment practices (Ginigaddarage et al., 2018). Placing the fish directly on the sand is more susceptible to the contamination by dust, insects, and sand (Bremner, 2002). In addition, the stability of microbial growth in dried fish depends on how much moisture they contain during processing and storage stages (Logesh et al., 2012). Rain and humid conditions hamper sun-drying by increasing the moisture content of dried fish and made them susceptible to blowfly larvae attacks (Kleih et al., 2003).

Techniques to Avoid the Contamination

The presence of microbes in dried fish presents a health hazard that needs to be prevented. To avoid the contamination of sand, dirt, and animals, the process of drying has to be done quickly using drying shelves, which can help to keep dried fish away from these sources of contamination (Ward & Beyens, 2015). Solar dryers, like greenhouse tunnel dryers and forced convective solar dryers can also help to avoid physical and atmospheric-driven contamination and they can accelerate the drying process (Sahu et al., 2016; Seerangurayar et al., 2019). These types of solar dryers are highly recommended as they protect dried fish from many sources of contamination like rain, humid environment, dust, insects and animals as well as increase the drying rate.

Conclusion

In this article, we verified the presence of microbiological contamination in two types of dried fish (anchovy and shark) available in local markets (Seeb and Buraimi). The major issue that affects the quality and safety of dried fish is the contamination caused by bacteria and fungi. Results from this study evidenced the presence of fungi and three species of bacteria; *E. coli, staphylococcus au-*

reus and coliforms. The microbial contamination in anchovy samples was more than in shark samples collected from the two local markets. To avoid the contamination mentioned above, we recommend using solar dryers for fish drying instead of open-sun drying, as they protect the dried fish from different types of contamination and speed up the drying process.

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