10 Laboratory Analysis

Abstract

Laboratory is a facility of scientific experiment in which samples are being tested in controlled conditions for the analysis of physical, chemical, and biological parameters that ensure quality of frozen shrimp and certify products either edible or not for human consumption. Laboratory analysis of export grade frozen shrimp is the determination of characteristics through a variety of tests to obtain its original properties. The chapter highlights the sample collection procedure for laboratory analysis and explains the details of analysis of physical, chemical (heavy metal, dye, pesticides, and antibiotics), and biological parameters of consumable export grade frozen shrimp items.

Keywords

Sample collection · Physical test · Chemical test · Biological test · Antibiotics test

Laboratory analysis of frozen shrimp is a mandatory process that must be performed before shipment. All tested parameters must be within standard limit. Products are shipped only based on satisfactory result of the laboratory analysis. The laboratory analysis procedure is completed through the following steps:

1. Sample collection
2. Testing/analysis
3. Reporting

Description of the above three steps is given below.

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10.1 Procedure of Sample Collection

Before going to start sample collection procedure, the following materials, equipment, and chemicals should be arranged. These are:

• Hand gloves, mask, and head gear	• Sealing machine
• Digital balance	• Polybag
• Hammer	• Sampling tag
• Sprit lamp	• Insulated box
• Knife	• Ice/dry ice/gel ice
• Forceps	• 70% ethanol
• Scissors	• Others

All necessary equipment used must be calibrated and sterilized. Samples for laboratory analysis are collected directly from processing industries. Sampling should be done after completion of the whole production. Partial productions are not allowed to collect the samples for laboratory testing. Samples should be drawn by maintaining standard sampling protocol (ISO) or buyer's specified method. All procedures must be in aseptic condition. The following procedure is maintained during collection of frozen shrimp:

- Samples for microbial analysis and chemical analysis should be drawn separately.
- Weight of samples are measured and recorded. Normally, 200 g sample for microbial analysis and 400 g sample for chemical and heavy metals analysis are enough for laboratory analysis.
- Sample must be sealed with air tide polybags.
- Sample identification tag is must.
- Sample is wrapped inside the insulated box with a clean and dry paper.
- Ice should be placed layer by layer, but a layer of ice at the bottom and top of the insulated box is mandatory.
- Standard icing ratio is 1:1, but quantity of ice may vary depending on sample volume and transport distance. Dry ice is better for long distance. Gel ice can also be used for sample transportation.
- Box should be wrapped with transparent tape and closed as early as possible.
- Lab address and test request form are added to the box and sent to lab for testing.
- A same volume of reference samples should be stored in supplier's cold storage as well for further analysis (if necessary).
- Frozen shrimp should be collected from each and every bag of the selected samples as per sampling plan. Sampling plan is made using following formula:

No. of cartons to be selected $= (\sqrt{n} + 1)/2$ $= (\sqrt{1800} + 1)/2$ $= 21.7$ $= 22$	Here, n = total no. of cartons = 1800

Table 10.1 Sample purchase order (PO) for a consignment

Production date	Product description	Brand name	Type of packing		Size and count of shrimp		Subtotal (MC)
			Gross weight	Net weight	8/12 (RC)	16/20 (FC)	
28.12.2021	Raw IQF BT HLSO shrimp	ABC	10 × 1 kg	10 × 800 g	400	–	400
29.12.2021	Raw IQF BT HLSO shrimp	ABC	10 × 1 kg	10 × 800 g	–	300	300
30.12.2021	Raw IQF BT PD shrimp	EFG	10 × 1 kg	10 × 800 g	500	–	500
31.12.2021	Raw IQF BT PDTO shrimp	EFG	10 × 1 kg	10 × 800 g	–	600	600
Grand total of master carton (MC)							**1800**

A total of 22 master cartoons have to be selected randomly for sampling. Sample selection procedure is also known as sampling plan. The details of sample selection procedure are given below (Tables 10.1 and 10.2).

Now,

Total no. of selected cartons will be 22

From 400 CTS of 8/12, RC, IQF Raw BT HLSO Shrimp (28.12.2021).

$$\text{Total no. of samples to be drawn} = \frac{\text{No of cartons to be selcted}}{\text{Total No.of catons on PO}}$$

$$\times \text{No.of variable(cartons)} = \frac{22}{1800} \times 400$$

$$= 4.8 \text{ or } 5 \text{ cartons}$$

At the same way:

$$\text{For 300 CTS of } 16/20, \text{FC, IQF Raw BT HLSO Shrimp} = \frac{22}{1800} \times 300$$
$$= 3.6 \text{ or } 4 \text{ cartons}$$

$$\text{For 500 CTS of } 8/12, \text{RC, Raw IQF BT PD Shrimp} = \frac{22}{1800} \times 500$$
$$= 6.1 \text{ or } 6 \text{ cartons}$$

Table 10.2 Final sampling plan

Production date	Product description	Brand name	Type of packing		Size and count of shrimp		Subtotal (MC)	No. of samples drawn
			Gross weight	Net weight	8/12 (RC)	16/20 (FC)		
28.12.2021	IQF Raw BT HLSO shrimp	ABC	10 × 1 kg	10 × 800 g	400	–	400	5
29.12.2021	IQF Raw BT HLSO shrimp	ABC	10 × 1 kg	10 × 800 g	–	300	300	4
30.12.2021	IQF Raw BT PD shrimp	EFG	10 × 1 kg	10 × 800 g	500	–	500	6
31.12.2021	IQF Raw BT PDTO shrimp	EFG	10 × 1 kg	10 × 800 g	–	600	600	7
Grand total of master carton (MC)							**1800**	**22**

$$\text{For 600 CTS of } 16/20, \text{FC}, \text{IQF Raw BT HLSO Shrimp} = \frac{22}{1800} \times 600 = 7.3 \text{ or } 7 \text{ cartons}$$

[Note: Sampling plan may change as per buyers' instruction/protocol/sampling plan]

Perform the following exercise

Exercise 1:	Make a sampling plan for the following breakdown: BT HLSO 70% NW 16/20 IQF 1000 CS BT HLSO 80% NW 21/25 IQF 1000 CTS
Exercise 2:	Make a sampling plan for the following breakdown: BT HOSO 16/20 S.IQF 600 CTS BT HOSO 26/30 S.IQF 800 CTS BT HLSO 21/25 IQF 400 CTS
Exercise 3:	Make a combined sampling plan for the following breakdown: BT HOSO 16/20 & 21/25 S.IQF 300 CTS & 600 CTS BT HLSO 8/12 & 13/15 IQF 400 & 600 CTS

- **Dry Ice and Gel Ice**

Dry ice and gel ice function as cooling agents and are used in long transportation of frozen sample. Both dry ice and gel ice are used as alternative to cube/regular ice without the messes of melting water. In the case of dry ice, insulated box must be sealed in such a way that no gases pass out of the box as they turn into carbon dioxide gases directly rather than liquid. Dry ice is a frozen carbon dioxide (CO_2) or solid form of carbon dioxide (CO_2), and it has no residue like cube/regular ice. Temperature of dry ice is much lower than that of cube/gel ice. Surface temperature of dry ice is −109.3 °F or −78.5 °C. Again, the temperature of gel ice is around 0 °C (32 °F) or lower. Gel packs are made of leak-proof polyethylene bags filled with cooling gel. The gel packs are often made of nontoxic materials, have no mess of melting water, and are free of contamination. Gel ice can be reused several times through freezing and thawing. The following are the images of sample collection procedure (Figs. 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, and 10.9).

[Note: Handling of dry ice should be done very carefully. This extreme cold ice is very dangerous if handling it without any protection. It may cause burn in your hands/body if it is used without any protection.]

10.2 Testing/Analysis

International standards are followed for **testing of seafood items**. **Testing** results confirm whether these products are safe for human consumption or not. **It**'s one of the greatest challenges in international food business. The main outcome of seafood testing is to confirm quality products, food safety, and consumer's satisfaction. It's a mandatory requirement for all seafood processing industries to confirm laboratory

Fig. 10.1 Sample isolation

Fig. 10.2 Sample collection

Fig. 10.3 Weighing of sample

test (physical, chemical, biological, heavy metals, dyes, pesticides, preservatives, additives, etc.) before shipping the consignment. Sometimes buyers perform their testing through other facilitated **independent** laboratories of the same country or outside of the country to make a comparison for more accurate results. Remember that we are dealing with food products, and our prime concern is food safety and quality. Any kind of hazards may cause serious illness in human health.

Fig. 10.4 Sample sealing

Fig. 10.5 Bottom layer of dry ice

Fig. 10.6 Layer of sample

Fig. 10.7 Final layer of dry ice

Fig. 10.8 Test sample

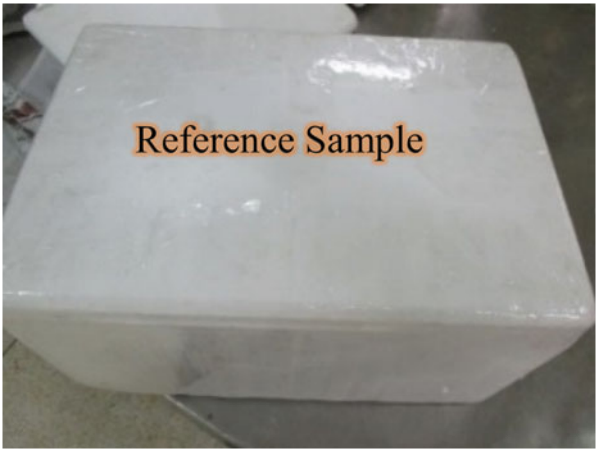


Fig. 10.9 Reference sample

10.2.1 Physical Test

Physical test is the first step of laboratory analysis. The following physical characteristics are tested in frozen shrimp:

- General appearance
- Freshness
- Texture
- Color
- Smell/odor
- Physical damage (clamps, glaze, broken pcs, etc.)

10.2.2 Biological Test

Spoilage of food is the degradation of color, texture, flavor, taste, as well as the nutritional value of products due to the activities of microorganisms. Microbial tests significantly contribute to the identification of pathogen and food spoilage microorganisms causing foodborne illness in human. Different types of spore-forming or non-spore-forming bacteria are responsible for food spoilage.

The following biological characteristics are tested in frozen shrimp:

- *Shigella* sp.
- *Salmonella* sp.
- *Bacillus cereus*
- *Vibrio cholerae*
- *Escherichia coli*
- *Vibrio vulnificus*
- *Vibrio parahaemolyticus*
- *Clostridium perfringens*
- *Listeria monocytogenes*
- *Staphylococcus aureus*
- Aerobic plate count
- Enterobacteriaceae
- Coliforms/total coliforms/fecal coliforms

10.2.3 Chemical Test

Different types of food additives, preservatives, antioxidants, sweeteners, salt, food colors, etc. are used in food to enhance the quality, safety, and shelf life of products. Chemical tests in frozen shrimp helps to assess the presence of chemicals and their concentration and summarize whether the food product is safe for consumption or not. The following chemical parameters are tested in frozen shrimp:

- Agar
- Indole
- Chlorine
- Citric acid
- Histamine
- Salt as NaCl
- Purity of salt
- Crude protein
- Monophosphate
- Diphosphate
- Triphosphate
- Polyphosphate
- Phosphate residue as P_2O_5
- Metabisulfite as SO_2
- Sulfite residues as SO_2
- Total volatile base nitrogen

The following dyes are tested in frozen shrimp:

- Crystal violet
- Malachite green
- Leuco-malachite green
- Leuco-crystal violet
- Other synthetic colors in food

10.2.4 Pesticide Test

Pesticides are used in shrimp processing industries for pest management. Sometimes buyers ask to test different types of pesticides. The following pesticides are tested in frozen shrimp:

- Ethoxyquin
- Trifluralin
- Ivermectin
- Chlorpyrifos
- Pendimethalin
- Organochlorine
- Ethoxyquin dimer
- Organochlorine pesticide residues
- Organophosphorus pesticide residues

10.2.5 Heavy Metal Test

The metallic chemical element which has relatively high density and is toxic at low concentrations is referred to as heavy metals (Pandey and Madhuri 2014). Pb, Hg, Cd, Cr, Cu, Zn, Mn, Ni, Ag, etc. are known as heavy metals. Among them the heavy metals As, Cd, Pb, and Hg are considered as most toxic to environment, animals, fishes, and humans (Pandey and Madhuri 2014). Chemical water pollution is the main source of heavy metal contamination. Heavy metals have the ability of bioaccumulation and biomagnification and can't be eliminated from the body by metabolic activities (Elbeshti et al. 2018). Consumption of heavy metal-contaminated fish is a risk for human health that may cause different types of health hazards like skin lesions, nerve damage, skin cancer, etc. Heavy metals in frozen shrimp are tested before shipping the consignment. The following heavy metals are tested in frozen shrimp:

- Arsenic (As)
- Cadmium (Cd)
- Mercury (Hg)
- Copper (Cu)
- Lead (Pb)

10.2.6 Antibiotics Test

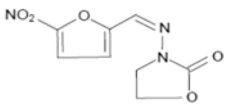
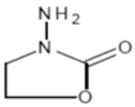
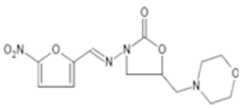
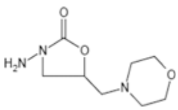
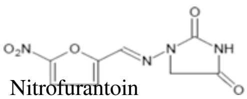
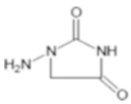
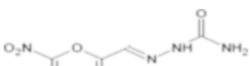
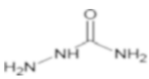
Antibiotics are medicine that slow down the growth of microorganisms or destroy it. Uses of antibiotics are strictly restricted in shrimp farms because of bioaccumulation. Experts are now concerned about resistance of antibiotics in human health. Resistance of antibiotics is the result of overusing and inappropriate using of antibiotics. Bioaccumulation can happen in youth to adult stage but significantly higher in youth stage and weakest during the adult stage of shrimp. The antibiotics exhibited higher bioaccumulation capacity in lipid-rich tissues especially head and gill of shrimp than muscle (Zhang et al. 2021). Antibiotics in frozen shrimp are tested before shipping the consignment because of mandatory requirements.

The following antibiotics are tested in frozen shrimp:

- Quinolones
- Trimethoprim
- Chloramphenicol
- Ciprofloxacin/enrofloxacin
- Nitrofurans metabolites (AOZ, AMOZ, AHD, SEM)
- Tetracycline (oxytetracycline, chlortetracycline, tetracycline)
- Fluoroquinolones (enrofloxacin, ciprofloxacin, sarafloxacin, difloxacin)
- Sulfonamides (sulfadiazine, sulfadimidine, sulfamethoxazole, sulfadimethoxine, sulfachloropyridazine), etc.

Nitrofurans are a broad-spectrum antibiotic, which works well against gram-positive and gram-negative bacteria. The tissue-bound residues of nitrofurans are very stable and do not degrade to a significant extent while preparing food like cooking, baking, grilling, and microwaving. Residues of nitrofurans ingested by consumption of contaminated product are bioavailable. When consumed, nitrofuran residues are absorbed by the consumer's body and form again tissue-bound residues. Parent molecules of nitrofuran are rapidly metabolized by animals, and their in vivo stability is no longer than a few hours, and they formed as persistent protein-bound residues. Unlike the parent molecules, these protein-bound metabolites are stable and persist in the body. The compound is considered carcinogenic and genotoxic; consumption over time of product contaminated with nitrofurans may cause human health risk. Nitrofurans were commonly employed as feed additives for growth promotion in livestock and aquaculture (i.e., fish and shrimp). The nitrofurans are a group of synthetic broad-spectrum antibiotics, which have been widely and effectively used for prophylactic and therapeutic treatment of bacterial and protozoan infections such as gastrointestinal infections caused by *Escherichia coli*, *Salmonella* sp., *Mycoplasma* sp., *Coccidia* sp., and coliforms (Vass et al. 2008).

There are four major nitrofurans: *furazolidone*, *furaltadone*, *nitrofurantoin*, and *nitrofurazone*. These nitrofurans are banned in EU because of their toxic and suspected carcinogenic and mutagenic properties (Commission Regulation 1442/95). In 2003 a definitive MRPL (Minimum Required Performance Limit) was set at 1 ng/g in the EU for the abovementioned four nitrofurans in poultry and aquaculture products (Commission Decision 2003/181/EC). The following structures of nitrofuran metabolites are tested in laboratory:

Parent compound to metabolites	Parent compound	Metabolites
Furazolidone to (3-amino-2-oxazolidinone (AOZ)	 Furazolidone	 AOZ
Furaltadone to (3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ)	 Furaltadone	 AMOZ
Nitrofurantoin to (1-aminohydantoïne (AHD)	 Nitrofurantoin	 AHD
Nitrofurazone to semicarbazide (SEM)	 Nitrofurazone	 SEM

Vass et al. (2008)

- **Others**

The following parameters are also tested sometimes as per buyer's requirement:

- Water content/moisture %
- Toxin (mycotoxins, phycotoxin, aflatoxins)
- Genetically modified organism (GMO)
- Fatty acid profile (saturated/unsaturated)
- Nutritional profile (carbohydrates/protein/fat/vitamins/ash, etc.)
- Shelf life, etc.

[Note: All parameters are not tested for every consignment. Some are mandatory (*Salmonella* sp., antibiotics) for every consignment, and some are occasional (pesticides, chemical). It depends on exporting country and customers' demands.]

References

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