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Safety of Fermented Foods

Assessing risks in fermented food processing practices and advice on how to mitigate them

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2 | Starter cultures & fermented food standards

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Additional fermented food guidance can be accessed at:
http://www.bccdc.ca/health-professionals/professional-resources/fermented-foods

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Section 3 | Food safety reviews of fermented foods

A national working group of health inspectors, food safety specialists, and industry fermentation experts reviewed this food safety guidance.

Each fermented food review includes:

- background on the food,
- a description of the food preparation,
- a food flow chart,
- a review of the potential issues with the food preparation, and
- food safety control points.

Foods covered in this guidance are sorted in order of increasing complexity and fermenting agent.

Figure 1 | Fermented foods described by fermentation agent and complexity

Complexity	Foods	Fermenting Agent	Section
high	Sausage	Added LAB[1], spontaneous moulds & yeasts	3.13
	Kefir, Kombucha	SCOBY[2] based: *Acetobacter*, yeast & mould	3.11-3.12
	Koji, Miso	*Aspergillus*, spontaneous or added yeast & LAB	3.10
	Tempeh	*Rhizopus*	3.9
	Natto	*Bacillus*	3.8
	Yogurt, Plant-based cheese	Added LAB	3.6-3.7
	Dosa, Idli, Fesikh	Spontaneous LAB and Yeast	3.4-3.5
low	Vegetables, Sauerkraut, Kimchi	Spontaneous or added LAB	3.1-3.3

[1] – LAB-lactic acid bacteria; [2] – SCOBY-symbiotic culture of bacteria and yeast

A non-fermented, high alkalinity processed food is also included in this guidance: pidan century egg (Section 3.14).

Box 1 | How to use the information in this food safety review

The information presented here lays out best practices for a variety of fermented foods, however, it does not replace or supersede federal and provincial guidance or regulatory requirements for fermented foods. Health inspectors, food safety staff, owner and operators of food processing facilities should follow federal and provincial food safety requirements. This work intends to assist food safety staff (health inspectors) to evaluate the safety of fermented foods and fermentation processes encountered during inspections. Owners and operators of food processing facilities may also find this guidance helpful as it reviews critical control points and measures recommended to produce safe fermented foods. The best available evidence guided this work at the time of publication. The application and use of this document is the responsibility of the user.

Guidance does not include information about good manufacturing practices, labelling practices, or management control programs for cleaning and sanitation, pest control, employee training etc. It is expected that operators will follow approved guidance and seek this information elsewhere.

3.14 | Pidan/Century egg/Hundred-year egg

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Overview

Description	 Pidan, also known as century or 100-year egg, is an alkali-treated fresh egg (usually duck egg). During alkalinization (also referred to as alkaline fermentation), with the aid of chemicals, egg proteins are hydrolyzed into amino acids resulting in the release of ammonia, an increase in pH and a decrease of a_w of the final product. Pidan has a strong hydrogen sulfide and ammonia smell. The egg white or albumen changes colour to a brown or greenish brown, with a gel-like texture; the egg yolk changes colour to blackish green, grass green or dark brown. The colour change has been partly contributed to the Maillard reaction between the glucose of the albumen and amino acids.
Starter culture	None. This is a chemical process, where the eggs are processed in an alkaline reagent such as sodium or potassium hydroxide (NaOH or KOH). NaOH is formed from the reaction of sodium carbonate (Na_2CO_3), water (H_2O) and lime (CaO) in the pickle solution or coating mud.
Key features	• Use of a validated recipe is recommended. • Ensure final a_w <0.92. A pH of greater than 9 is recommended. • If the pH of the final product is >9, the pidan may be stored at room temperature, otherwise, if pH is ≤9 or below the pidan should be refrigerated.
Hazards of concern	Chemical Hazards: • Heavy metal salts such as lead oxide, copper sulphate and zinc oxide are often used to reduce fermentation time, and may be present in century eggs. Biological Hazards: • Pathogens cannot grow in the final product due to low a_w and high pH, however, if pidan is contaminated, pathogens can survive the chemical process. No documented foodborne illness associated with pathogens and pidan have been identified.
Important control points	• Refrigerate raw eggs. • To limit microbial contaminants raw eggs must be cleaned by rinsing off any visible contamination or soil with potable water, then dried. Use only eggs with intact shells. Use graded eggs if available (e.g. duck eggs are not graded in Ontario and New Brunswick). • To limit chemical contaminants, avoid using heavy metal oxides such as lead oxide and zinc oxide to accelerate fermentation. • Ensure pidan has an a_w <0.92. If pidan is stored at room temperature ensure it has pH >9.0. Use of validated recipes can assist in obtaining required a_w and pH.

Background

Pidan, also known as century egg, alkaline egg, preserved egg, hundred-year egg or thousand-year-old egg, is an alkaline fermented ready-to-eat egg, made from duck, chicken or quail eggs.[1,2] Unlike other alkaline fermented foods such as natto, in which fermentation occurs with the aid of microorganisms or culture, pidan is produced using chemicals in an alkali-treated fermentation or alkalanization.[3] Pidan production dates back more than 600 years in China, and is a popular food item in China, East Asia and Southeast Asia. Pidan visibly differs from raw or hard cooked egg. The albumen or egg white in pidan becomes a brown or greenish brown gel, and the egg yolk colour can vary in shades of blackish green, grass green or dark brown.[1] The colour change has been partly contributed to the Maillard reaction between the glucose of the albumen and amino acids.[4] Pidan has a strong hydrogen sulfide and ammonia smell.[1]

Microbial growth and survival in pidan. Microbial growth in pidan is limited when it is properly produced; maintaining a high (alkaline) pH and an a_W < 0.92 are the key elements in the microbial safety of this product. The pH of pidan varies in published reports but is generally in the range of 9 to 11.5 and alkaline. Zhao et al. reports the pH of pidan to be around 11.3 (± 0.2).[10] Chang et al. reported a pH of 11-12 for egg white of pidan after 20 days of fermentation,[11] Zhang et al. reported a pH of 11.16 for pidan egg white that has undergone alkaline fermentation using potassium hydroxide or KOH,[12] and Wang J. et al. reported a final pH of 9 for pidan.[3] The University of Guelph's fact sheet on century eggs states that the pH can range from 9 to 9.5, with the pH of the final product being affected by the type of egg and the type of alkaline chemical reagents used.[3]

Salmonella spp., *B. cereus*, *B. maceranse*, *Stahylococus conhnii*, *Staphylococus epidermidis*, *Haemolyticus*, and *Staphylococus warneri* are the predominant microorganisms of concern in pidan.[13] The maximum pH value permitting the growth of *B. cereus* is 8.8. Optimum growth pH of common enteric microorganisms varies from 6 to 8.[14] In a study done by Fu Y.M and Su T., duck eggs were inoculated with two strains of *S. enterica serovar Enteritidis* (SE). During the processing, as the pH value of the yolk increased from 6.5 to 10.3, the viable *Salmonella* counts in the pidan decreased. Further decrease of viable cells were observed during the 3 month post-processing storage period. However after 3 months of storage *S. enteritidis* could still be detected in 40% of samples tested.[15] This study concluded that if the eggs are contaminated with *Salmonella*, the pathogen can survive in the fermented product.

Outbreaks and Recalls

No recalls of pidan were identified in Canada[16], the US[17], Australia and New Zealand[18]. Two recalls of imported fermented duck eggs due to copper residues above the maximum residue limit (MRL) was found in Europe;[19] and are described in Table 1. Although not stated in the recall, copper sulphate may have been used to accelerate the curing process leading to toxic copper concentrations. The recall did not specify the copper concentration, however The European Food Safety Authority (EFSA) has established an acceptable daily intake (safe level) of 0.07 milligrams per kilogram of body weight for the adult population for copper.[20]

Table 1 | Recalls and alerts related to pidan in Canada and elsewhere

Year	Hazard Category	Hazard Detail	Number Recalls	Country	Product Description
2023[21,22]	Chemical: copper	Copper - residue level above MRL	2	Denmark, Netherlands, France, Germany, Austria, Belgium, Portugal, and Spain	Fermented duck eggs imported from China

While pathogens such as *Salmonella* spp. have been isolated from eggs and poultry farms,[5-7] a search of published foodborne illness outbreaks associated with consumption of pidan in the Public Health Agency of Canada's Publicly Available International Foodborne Outbreak Database[8] and the Center for Disease Control and Prevention (CDC) outbreak database (NORS Dashboard)[9] did not identify any illnesses or outbreaks associated with this product.

Description of food preparation for pidan

Pidan is typically made from alkali-treated fresh duck eggs although other types of eggs, such as those from chickens and quail may also be used. During alkalinization (also referred to as alkaline fermentation), protein is hydrolyzed into amino acids, resulting in the release of ammonia and subsequently an increase in the pH of the final product.[3,10]

There are many preparation methods for pidan, however, when alkalinization is completed, all pidan eggs should have translucent, brown albumen, dark-green creamy yolk with a final a_W <0.92 and preferred pH >9.[1] The preparation steps, described below, are to wash eggs, coat eggs in the alkaline solution or paste, store eggs until final aw <0.92 is achieved, and to coat eggs. The final pH will determine whether the eggs may be stored at room temperature (pH >9) or refrigerated (≤9). The processing time varies depending on the chemical reagent used and the concentration of the chemicals used. Traditionally, raw eggs were coated in a paste made of tea leaves, lime, salt, clay and ash; then rolled in rice hulls or tea leaves and stored away for months to ferment. Modern methods soak raw eggs in a strong alkaline solution to shorten the processing time.[1] Acid pre-treatment of eggs (e.g. soaking eggs in 5% acetic acid for 30 min) can further reduce processing times.

Handling and purchase of eggs: In Canada, eggs are obtained and kept at 4°C and lower until processed. Eggs are washed and dried to remove visible contamination and soil, such as debris and dirt. It is recommended to use fresh, graded eggs (when possible) and to avoid using eggs that are cracked or visibly soiled. Eggs that are not fresh can result in a longer gelation time.[3,4] Eggs are considered to have an acceptable level of contamination when the shell does not have more than three stain spots, and the aggregate area of soiling does not exceed an area equivalent to more than 25mm^2 dirt and stains.[23]

Alkalinization of eggs: Eggs are added to the alkaline pickling solution or coated in the alkaline paste. Validated recipes capable of achieving an a_W of less than 0.92 should be used.

In the **traditional method**, eggs are coated/rolled in a paste made of salt, lime, ash, and liquid tea or water. Eggs are coated with a paste made of 125g sodium carbonate (Na_2CO_3), 65g of wood ash, 1 kg of calcium oxide (CaO), 100 g of salt (NaCl) and 500 ml of tea or water, or enough to coat 100 duck eggs. The surface is coated to obtain a paste thickness of 1 cm. They are then rolled in food-grade rice hulls and placed in enclosed jars at room temperature (20-25°C). The a_W is checked after 30 days to ensure it is less than 0.92, if not the process is continued for up to 5 months. If a_W fails to drop to ≤0.92 within 5 months, the batch should be discarded. Alternatively, a pickling solution can be made using the same ingredients, replacing wood ash with 16.25 g of calcium carbonate ($CaCO_2$). Add enough tea/water to obtain a liquid consistency. The egg can be immersed in the solution at room temperature for 45 days. Following this process eggs are washed and air dried.

The **modern method** has a shorter processing time because raw eggs are soaked in the strong, alkaline NaOH solution. In the traditional method NaOH is produced from mixing of lime and sodium carbonate but the sediments from the mixture block the egg-shell pores and lower the penetration rate of NaOH into the eggs, prolonging the process. A scan of validated modern method recipes did not find upper or lower limits for salt, cations and NaOH. One example recipe from peer reviewed papers lists chemical concentrations (w/v) as: 4.2% NaOH, 5% NaCl and 2% Chinese tea and 0.2% different cations (e.g. $CaCl_2$).[3,4,24-26] Soaking eggs directly in the lye solution can lower the production period to 14 days.[4] Acid pre-treatment of eggs, e.g., soaking eggs in 5% acetic acid for 30 minutes at room temperature, before soaking the eggs in alkaline solution can enhance the penetration and further reduce the production time. The process is completed when an a_W <0.92 is obtained. Salmonella, a common pathogen associated with raw egg, cannot grow at a_W <0.92.

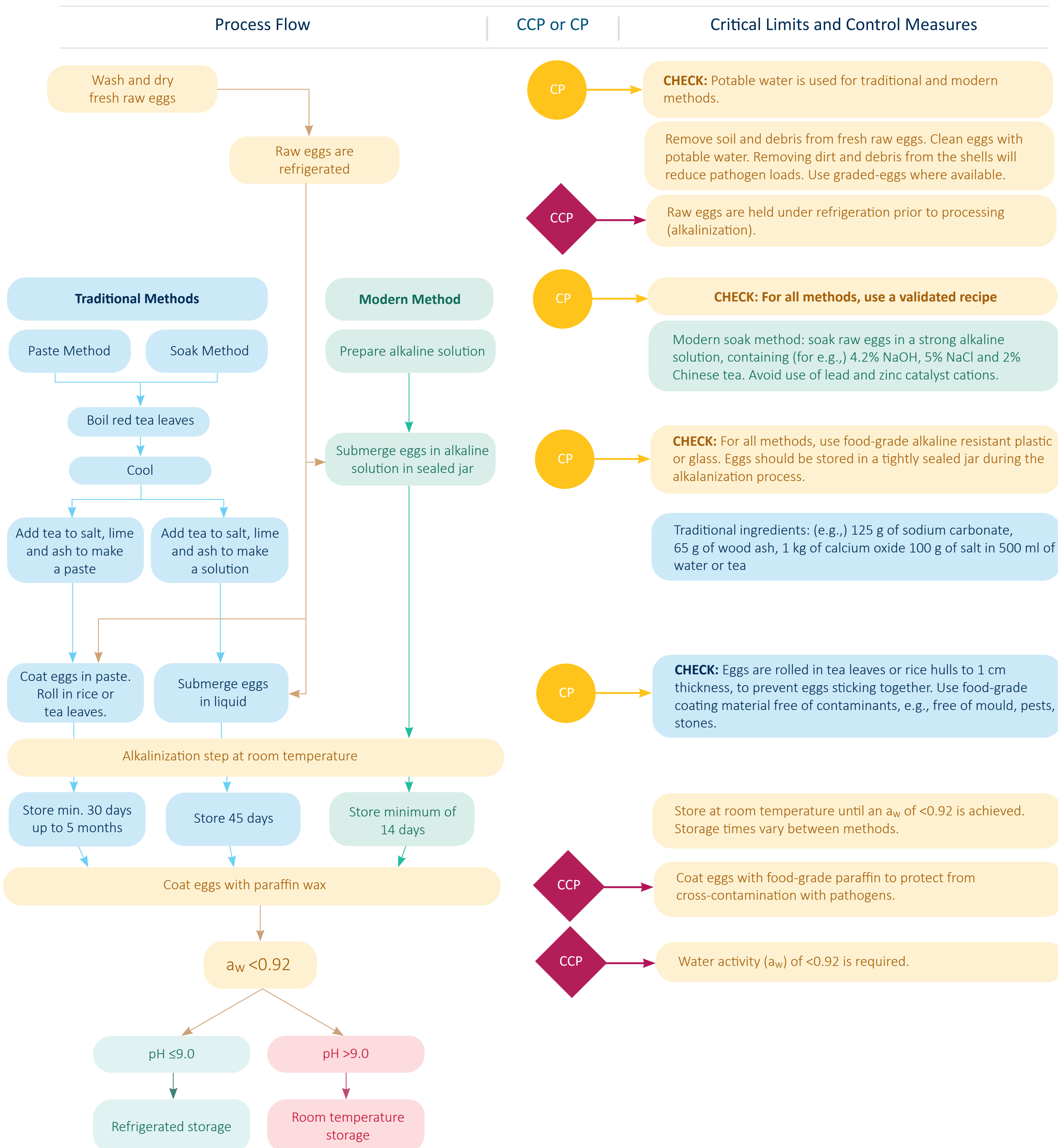
For traditional and modern methods, when alkalinization is completed, eggs are coated. The coating materials varies and includes food-grade paraffin (most commonly used) and rice hulls. Coating materials should be food-grade and free of contaminants (e.g., mould, insects). Coatings prevent cross-contamination with spoilage microorganisms and prevents dehydration of pidan albumen.[4] This process step is a CCP.

Sodium hydroxide (NaOH) is the main alkaline chemical reagent used in both traditional and modern methods, however other alkaline chemicals such as potassium hydroxide (KOH) have also been used in production of pidan. NaOH is formed from the reaction of sodium carbonate (Na_2CO_3), water (H_2O) and lime (CaO) in the pickle solution or coating mud. NaOH penetrates the eggshell and causes the degradation of egg protein, gelation and colour change. NaOH also releases hydrogen sulfide and ammonia, increasing the pH value of the final product. The gelation of egg white is due to proteolysis and the colour change results from the formation of ferrous sulfide (FeS).[3] The brown colour of the albumen is caused by the tea, also the crystallization of the degraded protein products results in the formation of snow-flake patterns in the albumen.[1]

Alkaline additives containing heavy metals, such as lead oxide (PbO) and zinc oxide (ZnO), are known to be used in production of pidan to accelerate the fermentation process.[27] However, their use may result in the presence of heavy metals such as lead in pidan.[3] According to Health Canada, lead is not permitted to be added to foods sold in Canada and exposure to even small amounts of lead can be hazardous to health.[28] Metal compounds containing heavy metals such as zinc and lead should not be used as their risks to health outweigh any potential benefit as fermentation agents.

We identified concerns with chemical contamination of pidan. A survey of heavy metal content in 35 preserved eggs in China concluded that the ingestion of this product should be controlled as it can be a source of lead (Pb), zinc (Zn) and copper (Cu) for consumers of this product.[29] In 2013 a number of companies producing preserved eggs were closed by authorities in China due to heavy metal contamination of their products. It was alleged that copper sulphate was used in production to shorten the curing period. Industrial copper sulphate usually contains heavy metals such as arsenic, lead and cadmium.[15] A 2017 study concluded that higher consumption of preserved eggs such as pidan is associated with depressive symptoms among adults in China.[30] However, no documented cases of foodborne illness were associated with pidan.

Pidan food flow chart | Process flow and controls



Potential issues with pidan food preparation

Chemical contamination, specifically heavy metal contamination, is the main concern with pidan production.

Issue	Description
Biological contamination	While we could not identify recorded foodborne illnesses associated with consumption of pidan, raw eggs contaminated with foodborne pathogens, e.g., *Salmonella* can survive in the product.[15] Use graded eggs (when possible), refrigerate raw eggs and wash and dry the eggs before processing to reduce the surface microbial contamination. Use food-grade rice hulls and paraffin.
Heavy metal contamination	Heavy metal salts such as lead oxide, zinc oxide, or copper sulphate solutions may be used by industry to reduce alkalinization time. However, their use may result in the presence of heavy metals (lead, zinc, copper) in pidan. Operators are recommended to use a validated recipe and avoid using alkaline additives containing heavy metals. According to Health Canada, lead is not permitted to be added to foods sold in Canada and exposure to even small amounts of lead can be hazardous to health.[28]
Chemical contamination	Use the recommended chemical and chemical concentration in the validated recipe to prevent chemical contamination of pidan. For example, coat eggs with a paste made of 125 g sodium carbonate, 65 g of wood ash, 1 kg of calcium oxide, 100 g of salt and 500 ml of tea or water. This is enough to coat 100 duck eggs to a thickness of 1 cm.
Prolonged processing time	Reduce processing time by using modern method of soaking eggs in a strong alkaline solution, of 4.2% NaOH, 5% NaCl and 2% Chinese tea and different cations for up to 14 days at 20-25°C. Acid pre-treat eggs (e.g. soaking eggs in 5% acetic acid for 30 min) can further reduce the processing time.[4] Use the recommended chemicals and chemical concentrations. Ensure the final product has a_W <0.92.
Prevention of processing and post processing contamination	Use potable water to wash eggs and make the coating paste or alkaline solution. Refrigerate the raw eggs. If the final product has a pH ≤9 pidan should be refrigerated. Coat the eggs. The coating material should be food-grade paraffin or rice hulls that are free of contaminants (e.g. no mould, insects etc.). The coating prevents cross-contamination of spoilage microorganisms.[4]
pH and available water	Ensure the final fermented product has a pH >9.0 and a_W <0.92 to limit the growth of pathogens.

Pidan food safety control points

Food safety points described in this section are shown in point form below:

- Refrigerate raw eggs.
- To limit microbial contaminants, raw eggs must be cleaned and dried; rinsed with potable water to remove soils and debris. Use graded eggs when possible without cracks or visible soils. Refrigerate washed eggs if not using right away.
- To limit chemical contaminants avoid using heavy metal oxides such as lead oxide and zinc oxide to accelerate fermentation.
- Ensure the final fermented product has an a_W <0.92. Final pH >9.0 is recommended for room temperature storage, if pH ≤9, refrigerate pidan.
- Coating material should be food-grade and free of contaminants (e.g., no mould, insects etc.).

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