

Gastric Emptying Scintigraphy Egg Radiolabeling Efficiency Pre- and Post- Microwave Cooking

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Brief Title: GES Egg Radiolabeling Efficiencies

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ABSTRACT (350/350 words)

Introduction: The accuracy and reproducibility of nuclear medicine gastric emptying scintigraphy (GES) require strict adherence to the Society of Nuclear Medicine and Molecular Imaging (SNMMI) standardized protocol. The SNMMI standardized GES protocol contains precise instructions for meal ingredients and preparation. Previous research demonstrated many laboratories were using whole eggs in the test meal as opposed to the guideline-recommended liquid egg white and some laboratories attempted to radiolabel the egg by adding the radiotracer after cooking. This study aimed to document the labeling efficiency of ^{99m}Tc -sulfur colloid (SC) added to whole eggs before and after microwave cooking.

Methods: Whole eggs were mixed with ^{99m}Tc -SC before and after microwave cooking. The radiolabeling stability of the eggs was tested after 2 and 4 hours of incubation in hydrochloric acid (HCl) and simulated gastric fluid with HCl and pepsin.

Results: The experiment showed that no matter what the testing condition, radiolabeling by adding ^{99m}Tc -SC to whole eggs before microwave cooking resulted in a significantly higher labeling efficiency than radiolabeling when the ^{99m}Tc -SC was squirted on eggs after microwave cooking. This finding persisted over time with the pre-cooking radiolabeling method still significantly higher at 2 and 4 hours after the egg was placed for both gastric fluid mediums. Simulated gastric fluid with pepsin at 2 hours, the labeling was significantly higher at 73.3% when the radiotracer was added before microwaving and 43.3% when added after cooking ($p < .001$).

The results of this study further showed that when egg labeling efficiency testing was performed in HCl without pepsin, the labeling was less stable as compared to testing performed in a simulated gastric fluid with HCl and pepsin. In the HCl only medium, the labeling efficiency decreased significantly between 2 and 4 hours for both radiolabeling methods.

Conclusion: The results of this study demonstrated the addition of $^{99m}\text{Tc-SC}$ to whole eggs after cooking resulted in considerably inferior binding of the radiotracer to the eggs which deteriorated significantly over time. The study further demonstrated that radiolabeling efficiency results vary depending on whether HCl or HCl with pepsin was used to simulate gastric fluid. Radiolabeling stability decreased over time when HCl without pepsin was used. The findings emphasize the criticality of adhering to the standardized meal and preparation as alternate cooking methods have different radiolabeling efficiencies.

Key Words: gastric emptying scintigraphy, radiolabeling stability, guidelines, meal preparation

Gastric Emptying Scintigraphy (GES) is frequently performed in nuclear medicine to evaluate gastric motility (1). GES is accomplished by radiolabeling the solid or liquid component of a meal and then measuring the radioactivity in the stomach over time. It has become the gold standard test for measuring the rate of gastric emptying due to its noninvasive, physiologic, and quantifiable properties.

However, numerous factors such as patient status, test meal composition and preparation, image acquisition parameters, and the method of data analysis can impact the accuracy and reproducibility of the test's results. To reduce variation in test performance and results, a standardized GES protocol was needed. In 2009, the Society of Nuclear Medicine and Molecular Imaging (SNMMI) published *Procedure Guideline for Adult Solid-Meal Gastric Emptying Study 3.0* (2). The standardized protocol must be strictly adhered to in order to deliver reliable results.

The SNMMI standardized GES protocol contains precise instructions for patient preparation including medication withholding, blood glucose level and fasting state; the meal ingredients and preparation; radiopharmaceutical dose; image views and frequency; and image processing using the geometric mean, decay correction, and percent retention (2).

Of specific interest for this study, the requisite GES meal includes 118 mL (4 oz) of liquid egg whites such as Eggbeaters (ConAgra Foods, Inc), 2 slices of toasted white bread, 30 g of jam or jelly, and 120 mL of water (2). To prepare the meal, 18.5 – 37 MBq (0.5–1 mCi) ^{99m}Tc -sulfur colloid (SC) is added to the liquid egg whites, the eggs are beaten well, and cooked in a microwave or nonstick skillet. The egg whites with the ^{99m}Tc -SC should be stirred once or twice during cooking and cooked until firm. The

jelly is spread on the bread after it is toasted. The meal items can be eaten separately or as a sandwich along with consumption of the water.

Despite the publication of the standardized GES protocol in 2009, a study by Farrell et al. in 2017 found considerable protocol noncompliance (3). Specifically, their research showed 69.3% of laboratory protocols do not adhere to the standardized meal. Many laboratories (48.1%) used whole eggs instead of the obligatory liquid egg white. Numerous laboratories injected the radiopharmaceutical directly into the egg yolk. Anecdotally, Farrell et al. noted several laboratories asked the patient to bring scrambled eggs with them to their appointment, and the technologist then squirted the $^{99m}\text{Tc-SC}$ on the previously cooked eggs.

As these findings were unquestionably contrary to the advocated SNMMI GES standardized protocol, this study aimed to document the binding rate of $^{99m}\text{Tc-SC}$ added to whole eggs before or after microwave cooking.

MATERIALS AND METHODS

Radiotracer Labeling Method

Two radiotracer labeling methods were utilized during the study to prepare the eggs for the GES meal. The same cooking technique was used for both radiolabeling methods. In the first method (pre-cooking labeling), 71 uCi (2.6 MBq) $^{99m}\text{Tc-SC}$ was added to the whole egg and mixed well before microwave cooking. In the second method (post-cooking labeling), 84-89 uCi (3.0 MBq) $^{99m}\text{Tc-SC}$ was squirted onto the previously cooked eggs.

Cooking Method

One Egg-Land's Best® egg was transferred from its shell to a 4-ounce (118 mL) Styrofoam cup and beaten for 30 seconds until the yolk and egg white were well blended. The beaten egg (with and without $^{99m}\text{Tc-SC}$ as noted above) was cooked by microwave in the same Styrofoam cup that was used for mixing. The mixture was cooked to a firm consistency on high power for 45 seconds, with stirring at 15-second intervals.

Simulated Digestion Materials

The stomach has 2 digestive processes that were replicated in this study: chemical and mechanical (4). During, the chemical process, gastric fluid is produced in the stomach and the pH is between 1.5 and 3.0 (5). As human gastric fluid was not available for this research, simulated gastric fluid was used to mimic human digestion of proteins. The base of the simulated gastric fluid used in this experiment consisted of 3.5 mL of hydrochloric acid (HCl) (0.0847 M) combined with 3.06 g of sodium chloride (NaCl) (0.1044 M) in 500 mL of distilled water resulting in a pH of 1.5 (6).

In the stomach, hydrochloric acid (HCl) converts pepsinogen, which is also produced in the stomach, to pepsin. Pepsin breaks down proteins to peptides. Several previous studies evaluating the stability of radiotracer binding to the solid-meal component were conducted using only HCl. However, evaluation in HCl without pepsin may lead to misleading results as the meal may be stable in HCl but not in HCl with pepsin (7). For this reason, the study was conducted both without and with 0.1 g pepsin added to the test tubes.

Simulated Digestion Process

The microwaved eggs were cooled to room temperature and chopped to mimic chewing (Figure 1. B). Egg samples (labeled pre- and post-microwave cooking) weighing 0.5 g (Figure 1. A) were placed in 47 test tubes (6 per condition, however, one tube was damaged during the experiment and removed) containing 1.25 mL of HCl (Figure 2). Pepsin was combined with HCL for half of the test tubes. The tubes were then paraffin capped and placed in a water bath at 37°C with stirrers at 125 rpm for 2 and 4 hours to mimic mechanical and chemical digestion (Figure 1. C).

Radiolabeling Stability Testing

To separate the solid egg material from the liquid, a filter was created using a 3-mL syringe barrel and sterile gauze. The gauze was cut and folded into 1 x 1 in inch sections and packed into the barrel of the syringe (Figure 3. A). The gauze filter was then wetted with 0.5 mL of 1% bovine serum albumin in isotonic saline. The bovine serum albumin was used to prevent $^{99m}\text{Tc-SC}$ adhesion to the gauze (8). The bovine serum albumin rinse was followed by 1 mL of isotonic saline rinse.

To separate the solid material from the liquid in the test tubes, the egg and fluid were transferred to the previously prepared syringes with gauze at 2 and 4 hours after the start of the test (Figure 3. B and C). To ensure all material was transferred from the test tube to the syringe, the test tube was rinsed once with 1 mL of isotonic saline which was also filtered through the syringe.

The gauze with undigested egg material, the liquid filtered from the digestive solution, and rinse were counted in a well counter (Knight, 2007). The counted activity was background and decay corrected?

Labeling efficiency (LE) was determined by:

$$\text{LE(\%)} = \frac{\text{Gauze} + \text{Egg Counts (DPM)}}{\text{Total Counts (DPM) (Gauze+Egg+Filtrate+Rinse)}} \times 100.$$

Independent students t-tests were used to compare the labeling efficiency of the samples. Comparisons were made between labeling methods (pre-cooking labeling vs. post-cooking labeling), incubation time (2 vs. 4 hours), and whether digestion occurred in HCl or HCl with pepsin added (no pepsin vs. pepsin)

Results

Comparing the timing of the ^{99m}Tc -SC egg labeling – whether the tracer was added before after microwave cooking – showed that no matter whether the sample was counted at 2 hours or 4 hours and with or without pepsin added to the digestive solution, the radiotracer binding to the eggs was always significantly higher when the radiotracer was added before the eggs were microwaved. For example, in the plain HCl solution (without pepsin) at 2 hours, the labeling efficiency pre-cooking was 95.4% and post-cooking was 42.3% ($p < .001$). Similarly, in the digestive fluid with pepsin at 2 hours, the labeling was significantly higher at 73.3% when the radiotracer was added before microwaving and 43.3% when added after cooking ($p < .001$) (Table 1).

Comparing the stability of the radiotracer labeling over time showed that for simulated digestive fluid with just HCl (no pepsin), when the radiotracer was added before microwaving, the labeling efficiency of the eggs significantly decreased between

2 hours (95.4%) and 4 hours (78.0%) ($p=.01$). Similarly, when the radiotracer was added after microwaving, the labeling efficiency also significantly decreased at 2 hours (57.3%) and 4 hours (39.5%) ($p=.02$). However, when the digestive fluid contained both HCl and pepsin, radiotracer labeling efficiency did not change significantly between 2 hours and 4 hours no matter whether the radiotracer was added before (73.3% vs. 73.1%, $p=.94$) or after microwave cooking (43.3% vs. 35.0%, $p=.10$).

DISCUSSION

The accuracy and reproducibility of nuclear medicine GES require strict adherence to the SNMMI standardized protocol (1). A recent study by Farrell et al. found a majority of nuclear laboratories performing GES are not in compliance with the standardized protocol (9). In particular, almost half of the laboratories evaluated used whole eggs in the test meal as opposed to the requisite liquid egg white meal. In addition, Farrell et al. noted several laboratories attempted to label the eggs by adding the radiotracer after cooking. Both methods are contrary to the methods detailed in SNMMI standardized protocol.

This study aimed to document the labeling efficiency of $^{99m}\text{Tc-SC}$ added to whole eggs before and after microwave cooking. The experiment showed that no matter what the test condition, radiolabeling by adding $^{99m}\text{Tc-SC}$ to whole eggs before microwave cooking resulted in a significantly higher labeling efficiency than radiolabeling when the $^{99m}\text{Tc-SC}$ was squirted on eggs after microwave cooking. This finding persisted over time with the pre-cooking radiolabeling method still significantly higher at 2 and 4 hours after the egg was placed in the simulated gastric fluid.

The study finding that labeling efficiency was significantly higher when $^{99m}\text{Tc-SC}$ was added to eggs before microwave cooking was not surprising. It is known that $^{99m}\text{Tc-SC}$ fixes to albumin (egg white protein) which denatures as it cooks binding the $^{99m}\text{Tc-SC}$. Adding $^{99m}\text{Tc-SC}$ to previously cooked eggs does not allow the $^{99m}\text{Tc-SC}$ to bind to the albumin and then be denatured (10). The radiolabeling post-microwave cooking method range of 35.0% -56.3% was expected. Thus, the post-cooking method of radiolabeling, where the $^{99m}\text{Tc-SC}$ is squirted on eggs after microwave cooking, is not optimal and should not be used.

The results of this study further showed that when egg labeling efficiency testing was performed in a simulated gastric fluid containing only HCl, the labeling was less stable, and the labeling efficiency decreased significantly between 2 and 4 hours. This finding occurred for both radiolabeling cooking methods. However, when the simulated digestive fluid more closely mimicked real human digestive fluid including pepsin, the labeling efficiency remain unchanged between 2 and 4 hours. This finding indicates that stability testing in a gastric fluid with HCl only, without pepsin, may be misleading such that GES meal testing in only hydrochloric acid may not be as stable as in simulated gastric fluid with pepsin (7).

The results of this study are similar to the findings obtained by Knight et al. in 2007 (8). At 2 hours, Knight et al. found the labeling efficiency of whole eggs tested in hydrochloric acid (pre-cooking labeling) was 96.9% compared to our finding of 95.4%. In a similar experiment, Ertay et al. documented a labeling efficiency of 95%. In human gastric fluid, at 2 hours (9), the labeling efficiency obtained by Knight was 73.1%. The labeling efficiency in this experiment using simulated gastric fluid was 73.3%.

Incongruently, Knight et al. had different results at 4 hours in HCl testing fluid without pepsin. In their experiment, the labeling efficiency was not significantly different between 2 hours and 4 hours. At 2 hours the labeling efficiency was 96.9 and at 97.3 at 4 hours. However, in our experiment, the labeling efficiency tested in hydrochloric acid decreased significantly from 2 hours at 95.4% to 78.0% at 4 hours. Contrarily, in human gastric fluid, Knight et al. found the labeling efficiency decreased significantly from 2 hours at 73.1% to 42.5% at 4 hours. However, our results found the labeling efficiency remained unchanged with 73.3% at 2 hours and 73.1% at 4 hours.

The reason for the incongruent findings at 4 hours between our results and Knight's results is difficult to suppose. However, one possible explanation may be that Knight et al. used actual human gastric fluid obtained from subjects undergoing elective endoscopy. Our experiment used HCl with pepsin added to simulate gastric fluid.

Differences in experimental technique related to egg beating and mixing or cooked egg firmness may also explain the difference in results at 4 hours between our experiment and Knight's (8). Differences in binding stability could have been exacerbated by the use of whole eggs as opposed to recommended egg whites. ^{99m}Tc -SC binds to the albumin or egg white protein and is denatured and binding during cooking (10). Knight et al demonstrated the possible fluctuation while microwaving whole eggs, which allowed the albumin to separate from the yolks during the cooking process while compared to egg substitute. ^{99m}Tc -SC does not bind to egg yolks.

Strict adherence to the standard gastric emptying meal and preparation method is critical for accurate GES results. The ^{99m}Tc -SC must bind tightly to the egg forming the solid component of the meal (11). The gastric emptying rate of solids and liquids is

different. Liquids begin leaving the stomach immediately upon ingestion with an exponential rate of emptying. Solids enter the stomach and are broken down by mechanical contraction and gastric fluid. Solids empty more slowly following a lag phase and then at a fixed rate. If the $^{99m}\text{Tc-SC}$ is not tightly bound to the eggs (solid component) due to improper labeling, the study essentially becomes a liquid GES because part of the meal exits the stomach as a liquid. This creates the possibility of a false negative gastric emptying study.

Limitations

This study is somewhat limited as whole eggs instead of the recommended egg substitute were used to test radiolabeling stability. However, considering the findings by Farrell et al. that many laboratories use whole eggs and add the radiotracer after cooking, the results of this study using whole eggs and different cooking methods demonstrates unsatisfactory radiolabeling efficiency. This study is also limited in that the adequacy of mixing and cooking was not tested. However, Knight et al. imaged the cooking methods of whole eggs vs egg substitutes and noted the nonhomogeneous albumin and yolk during microwave cooking. Bonta et al. evaluated the quality of meal preparation and egg mixing between technologists by imaging the radiolabeled eggs before patient ingestion (12).

Conclusion

The accuracy and reproducibility of nuclear medicine GES are dependent on strict compliance with the SNMMI standardized protocol. This study documented the radiolabeling stability of $^{99m}\text{Tc-SC}$ added to whole eggs before and after microwave

cooking. The results showed the addition of ^{99m}Tc -SC to whole eggs after cooking resulted in inferior binding of the radiotracer to the eggs which deteriorated significantly over time. The study further demonstrated that radiolabeling efficiency results vary depending on whether HCl or HCl with pepsin is used to simulate gastric fluid. Radiolabeling stability decreased over time when HCl without pepsin was used. The results emphasize the criticality of adherence to the standardized meal and preparation as alternate cooking methods have varying radiolabeling efficiencies.

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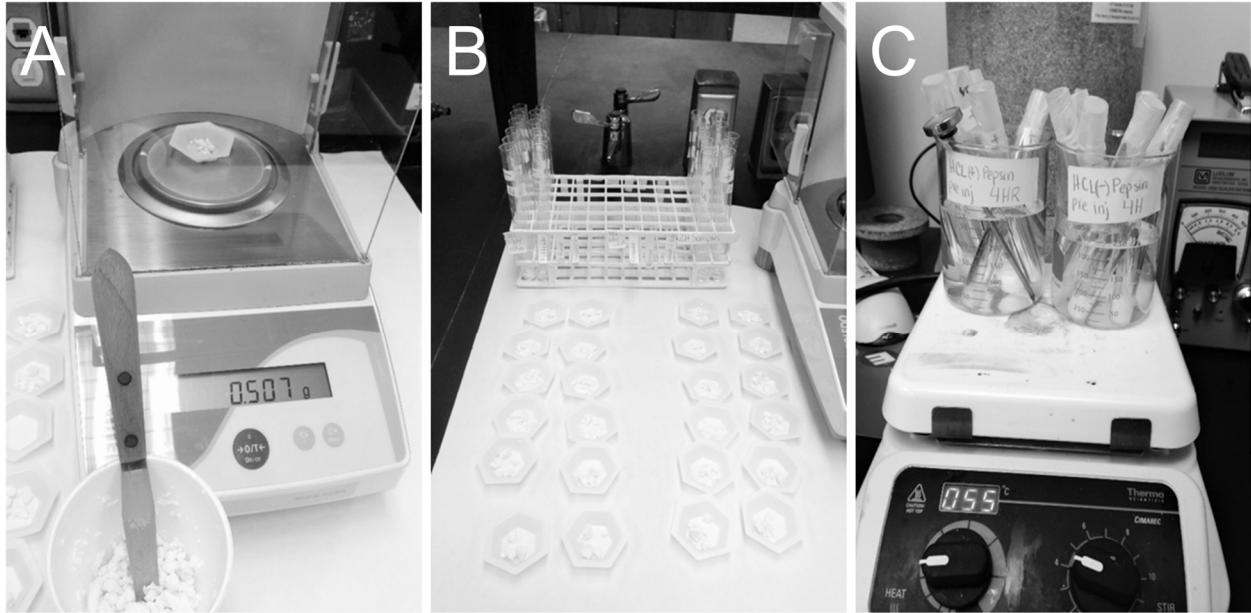
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A Standardized sample size

B Samples added to SGF

C Incubated at 37°C

Figure 1. Photographs showing (A) weighing method to assure standardized 0.5 g radiolabeled egg samples size (B) egg samples prior to being added to the simulated gastric fluid, and (C) 37°C water bath and stirring at 125 rpm.

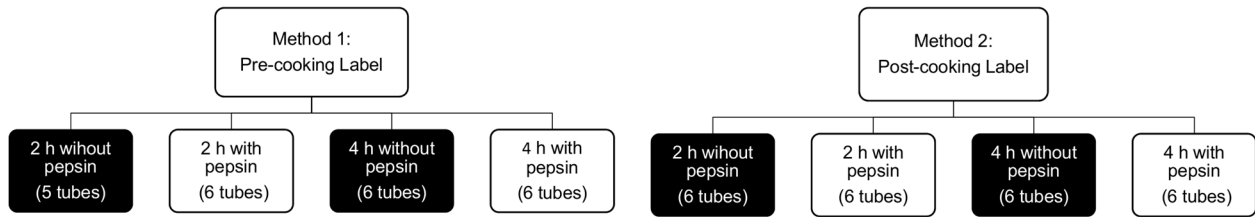


Figure 2. Egg labeling test conditions. Two methods were utilized to radiolabel whole eggs with ^{99m}Tc -sulfur colloid (SC). For Method 1, the ^{99m}Tc -SC was added to the beaten eggs prior to microwave cooking and for Method 2, the ^{99m}Tc -SC was squirted on the eggs after microwave cooking. The stability of the radiolabel was tested for both methods at 2 and 4 hours. The stability was also testing using a simulated gastric fluid with just hydrochloride and with hydrochloride and pepsin.

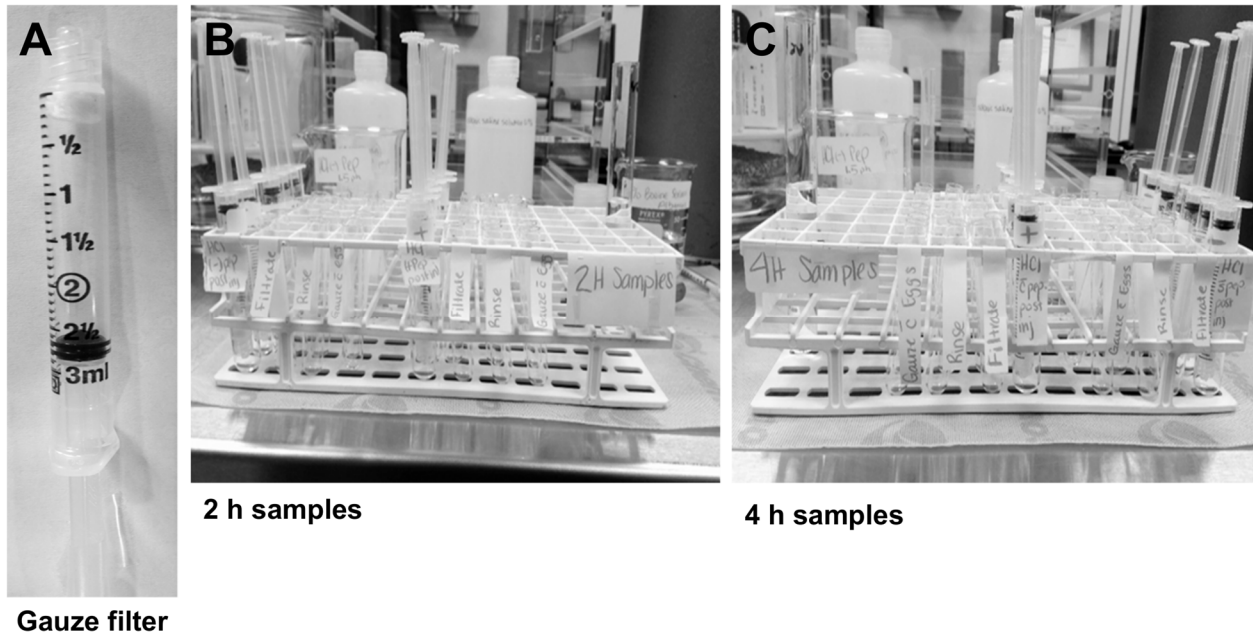


Figure 3. A) To test the stability of the radiolabeled eggs, a filter was created using a gauze and a 3 mL syringe. The gauze was wetted with 0.5 mL of 1% bovine serum albumin to prevent adhesion of the ^{99m}Tc -sulfur colloid to the syringe and gauze. B) and C) To separate the solid material from the liquid in the test tubes, the egg and digesting fluid was transferred to the previously prepared syringes with gauze at 2 and 4 hours after the start of the test.

Table

Table 1. Percentage of 99mTc-Sulfur Colloid Bound to Whole Eggs

No Pepsin			Pepsin				
	Pre-Cooking	Post-Cooking	p-value		Pre-Cooking	Post-Cooking	p-value
2 HR	95.4% (±2.4%)	57.3% (±8.4%)	<.001	2 HR	73.3% (±10.9%)	43.3% (±8.8%)	<.001
4 HR	78.0% (±11.5%)	39.5% (±10.4%)	<.001	4 HR	73.1% (±8.7%)	35.0% (±10.7%)	<.001
Pre-Cooking Labeling			Post-Cooking Labeling				
	2 HR Samples	4 HR Samples	p-value		2 HR Samples	4 HR Samples	p-value
No Pepsin	95.4% (±2.4%)	78.0% (±11.5%)	.014	No Pepsin	57.3% (±8.4%)	39.5% (±10.4%)	.019
Pepsin	73.3% (±10.9%)	73.1% (±8.7%)	.941	Pepsin	43.3% (±8.8%)	35.0% (±10.7%)	.097
Pre-Cooking Labeling			Post-Cooking Labeling				
	No Pepsin	Pepsin	p-value		No Pepsin	Pepsin	p-value
2 HR	95.4% (±2.4%)	73.3% (±10.9%)	.003	2 HR	57.3% (±8.4%)	43.3% (±8.8%)	.018
4 HR	78.0% (±11.5%)	73.1.0% (±8.7%)	.424	4 HR	39.5% (±10.4%)	35.0% (±10.7%)	.480

2 Hr = 2-hour incubation period; 4 Hr = 4-hour incubation period; No pepsin = hydrochloric acid digestive solution only; Pepsin = hydrochloric acid plus pepsin digestive solution; Pre-cooking = 99mTc-sulfur colloid added before cooking eggs; Post-cooking = 99mTc-sulfur colloid added after cooking eggs