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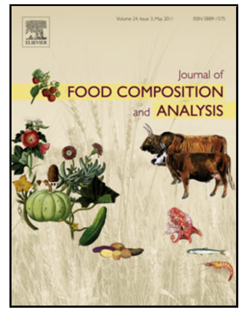

Content and Variability of Vitamin D and Iodine in Processed Egg Products in the United States (U.S.)

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Content and Variability of Vitamin D and Iodine in Processed Egg Products in the United States (U.S.)

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Highlights:

- Eggs, including processed forms, are an important source of vitamin D and iodine.
- Iodine, vitamin D, and 25-hydroxyvitamin D values in processed eggs are reported.
- Nutrient content of dried and frozen egg forms were similar on a dry-weight basis.
- Variability was seen in some dried and frozen yolk, white, and whole egg samples.

Abstract:

Iodine and vitamin D deficiencies among specific subpopulations within the U.S. are of concern. Eggs, including processed forms, can be an important dietary source of iodine, vitamin D_3, and 25-hydroxyvitamin D_3. Processed eggs (dried and frozen liquid forms of whole eggs, yolks, and whites) are used in food manufacturing and foodservice. Samples were obtained from six U.S. processors (up to three production lots per category), to examine analytical iodine and vitamin D values and variability measures. Samples and quality control materials were sent to USDA validated analytical laboratories. Nutrient values were statistically compared after adjusting the values for moisture content, with each nutrient dataset modeled separately using linear mixed models. No significant differences were found for vitamin D_3, 25(OH)D_3 or iodine content of dried eggs compared to frozen liquid eggs by their whole egg and yolk parts, on a dry weight basis. Updated values and variability data for processed eggs were incorporated into FoodData Central, the U.S. Department of Agriculture food composition database. These data contribute valuable information for estimating U.S. intake amounts of iodine and vitamin D.

Keywords: iodine; vitamin D; 25-hydroxyvitamin D; eggs; food analysis; food composition; nutrient data

1. Introduction

Eggs are an important dietary source of high-quality protein, essential lipids, and some vitamins and minerals (Wu, 2014). Significant iodine amounts have been observed in shell eggs (Ershow et al., 2018), according to analytical data from the Food and Drug Administration (FDA)'s Total Diet Study (Pehrsson et al., 2016), suggesting that eggs can be an important dietary source of iodine. Eggs have also been listed as a notable source of vitamin D [D_3 and 25(OH)D_3] considering the content and rate of reported consumption (Exler et al., 2013; Taylor et al., 2014). Per capita U.S. consumption was 284 eggs in 2018 (USDA, 2019a).

Iodine and vitamin D deficiencies have been observed in the U.S. and the world (Iodine Global Network, 2019; Palacios & Gonzalez, 2014), which are of concern among nutrition scientists. Iodine deficiency can lead to goiter and hypothyroidism, and adequate iodine intake is especially critical for maternal health and fetal development during pregnancy (Pearce, 2015). Iodine deficiency was common in the Great Lakes, Appalachian, and Northwestern regions of the U.S. (Pearce, 2007) until iodized salt was introduced in the 1920's. After that, iodine deficiency in those areas was reduced or eliminated (Pearce, 2015). However, since the 1970s, U.S. iodine intake has decreased by 50% (Pearce, 2015), and iodine deficiency has recently been observed in some population groups (Chow et al., 2015; Pearce, 2015).

Many reasons for low iodine intake or deficiency may be attributed. For example, a global strategy to combat iodine deficiency is fortification of salt with iodine, but iodized sale is not mandatory in the U.S. (Leung et al., 2012; Malouf et al., 2015). Although estimated iodized table salt sales in the U.S. are ~53% of all table salt (Malouf et al., 2015), with usage in ~87% of households (World Health Organization, 2007), some groups may consistently not consume iodized salt. The vast majority of U.S. salt intake is attributed to commercially processed foods, which typically are not formulated with iodized salt (or with other iodine-containing ingredients) (Pearce, 2015). Other types of salt — such as kosher and sea salt — are not commonly iodized but have increased in popularity (Pearce, 2015). Less than half of U.S. prenatal multivitamins contain iodine (Leung et al., 2009), even though nearly two-thirds of standard U.S. adult multivitamins contain iodine (Pehrsson et al., 2016). Additionally, salt consumption has decreased because of its association with cardiovascular risk (Pearce, 2015); moreover, food restrictions (e.g., vegan or vegetarian diets), severe food selectivity, and allergen-free diets (e.g., dairy or seafood) increase the risk of iodine deficiency (Labib et al., 1989; Leniszewski and Mauseth, 2009; Leung et al., 2011; Yeliosof & Silverman, 2018).

Vitamin D deficiency is a key worldwide public health concern (Hilger et al., 2014; Palacios & Gonzalez, 2014), including countries at low latitude where year-round sun exposure had been previously presumed sufficient to prevent deficiency (Palacios & Gonzalez, 2014). Deficiency has been observed in industrialized countries despite vitamin D fortification (Palacios & Gonzalez, 2014) including in the U.S., which has several foods which are routinely fortified (e.g., milk, cereals, juice; Hilger et al., 2014). Nearly 20% of Americans of ages 1 to 70 years are estimated to be "at risk" or deficient in vitamin D, based on serum 25(OH)D levels applied to National Health and Nutrition Examination Survey participant data (Manson et al., 2016). Many symptoms, such as bone pain, muscle weakness, fatigue, increased risk of falling, and cardiovascular disease, are related to vitamin D deficiency (Holick and Chen, 2008). Vitamin D deficiency has been reported to cause growth retardation and rickets in children; osteopenia, osteomalacia, and osteoporosis in adults (Holick, 2007).

Processed egg products, which are dried and frozen liquid forms of whole eggs, yolks, and whites (hereafter referred to as processed eggs), compose about 30% of total U.S. egg production (United Egg Producers, 2019; Wu, 2014). Processed eggs can be composed of shell eggs produced directly for this purpose, but are also an avenue for using eggs not suitable for the shell market (Cotterill & McBee, 1995) such as instances of shell egg production exceeding demand or eggs unsuitable for carton packaging (i.e. too small). Benefits of using processed yolks, whites, or whole eggs instead of fresh shell eggs in terms of food manufacturing include uniformity, stability, enhanced convenience and product quality, labor savings, improved storage requirements, and food safety. Processed eggs are used in commercial baked items, manufactured products such as ice cream and salad dressings, and a variety of foodservice applications (USDA, 2011; Wu, 2014), making them a potentially important contributor to dietary iodine and vitamin D.

With the addition of vitamin D to the Nutrition Facts Panel (Federal Register, 2018), food companies calculating nutrient profiles for their package labels needed up-to-date data on the vitamin D content of foods. However, prior to this study, U.S. data for iodine or 25(OH)D_3 in processed eggs were not available in the U.S. Department of Agriculture (USDA) food composition database, which has been known as the National Nutrient Database for Standard Reference Legacy (USDA, 2018). Data on iodine and vitamin D, especially 25-hydroxyvitamin D_3 [25(OH)D_3], in foods in the U.S. are lacking (Ershow et al., 2018; Roseland et al., 2017). Therefore, a research study was conducted to obtain analytical data for iodine and vitamin D, including 25(OH)D_3, in six categories of processed eggs (whole eggs, yolks, and whites, in dried and frozen liquid types), including a sampling plan that enabled estimation of variability within these six egg categories. Fresh retail eggs will be analyzed in a future study. The hypothesis of this study is that the vitamin D and iodine content of dried eggs compared to frozen liquid eggs do not significantly differ, when compared after values for both forms are adjusted for moisture content.

2. Materials and Methods

2.1. Samples

Samples were supplied by six major producers of processed egg products (designated processors U–Z), identified by the American Egg Board (Chicago, IL). No specific market share data were available. The producers were asked to supply samples (~0.5–1.8 kg each) of frozen liquid and dried whole eggs, egg yolks and egg whites from three different production lots during 2017. The sample request specified ‘plain’ products, with no added salt or sugar. Samples were obtained in August and September 2018. Three lots per processor were usually available, but not for all categories from all processors. Specifically, no frozen liquid yolks and only two lots of frozen liquid whole and dried whole eggs were available from Processor W, no dried whites and only two lots of dried yolks were available from Processor Y and no frozen liquid whole eggs and only one lot of frozen liquid whites were available from Processor X.

Samples were shipped to the Food Analysis Laboratory Control Center at Virginia Tech (Blacksburg, VA), where they were subsampled for analysis. Liquid egg samples were shipped either frozen on dry ice or refrigerated on ice packs. Samples received frozen were stored frozen (–20 ± 3 °C), and samples received refrigerated were stored refrigerated (4 ± 3 °C) upon receipt. Dried egg samples were shipped at ambient temperature and stored at 22 ± 3 °C until subsampled for analysis.

The total material received for each sample was homogenized and subsamples were taken for laboratory analysis prior to the "use by" date as follows. Frozen liquid egg products were thawed completely (generally overnight) in the refrigerator (4 ± 3 °C) then homogenized in a stainless steel bowl using a hand blender (Cuisinart® model CSB-1C). Dried egg products were thoroughly mixed in a stainless steel bowl. Immediately after homogenization, subsamples were dispensed uniformly into 30-mL pre-cleaned glass jars with Teflon™-lined lids (Qorpak, Bridgeville, PA), with the exception of dried eggs for analysis of proximates, which were placed into 60-mL Whirl-Pak® bags (Nasco, Fort Atkinson, WI). Each subsample was blanketed with nitrogen gas, capped, secured at closure with Scienceware™ label tape (Bel-Art, South Wayne, NJ), and stored at < –55 °C prior to analysis.

2.2. Nutrient analyses

Subsamples were shipped on dry ice to a laboratory that had been validated by USDA through use of AOAC or other validated methods. Validating the laboratory before analysis involved USDA's approval of the laboratory's methods, as part of the contractor qualification process (Haytowitz et al., 2008). Vitamin D (D_3, 25(OH)D_3, D_2), iodine, and moisture were analyzed. Table 1 summarizes the analytical methods (AOAC, 2017; Huang et al., 2019; Sullivan & Zywicki, 2012).

2.3 Quality control

Samples for each nutrient were analyzed in batches along with food matrix certified reference materials (CRM) and/or in-house control materials (CC) developed at FALCC for the National Food and Nutrient Analysis Program (NFNAP) to monitor the precision and accuracy of the results (Phillips et al., 2006). The CRM and CC were selected for each nutrient and egg category based on expected concentration of the analyte and similarity of the food matrix (e.g., proximate composition, fat content), and are listed in Table 2. In-house CCs included liquid whole eggs (Whole Egg CC), liquid egg whites (Egg White CC), vitamin D-fortified cheese (Processed Cheese CC), beef baby food (Beef Baby Food CC), flour (for moisture in dry eggs) (Flour CC), and a mixed food composite (Mixed Dish V CC). Certified reference materials were procured from the National Institute of Standards and Technology (NIST) Office of Reference Materials (Gaithersburg, MD) (NIST) and included SRM® 1845a Whole Egg Powder, SRM® 1549a Whole Milk Powder, and SRM® 1849a Adult/Infant Nutritional Formula.

After analysis, nutrient data for all samples and quality control materials were thoroughly reviewed by NDL scientists according to a data quality evaluation system (Phillips et al., 2006), including comparison of results for CRM to certified values to assure that results were within previously determined acceptable ranges.

2.4. Statistical Analyses

The least squared mean and standard error (SE) for each nutrient in each egg category were calculated and reported as amounts per 100 grams (Table 3). The mean and standard deviation (SD) per nutrient per processor for each category were calculated. Thus, most of the nutrient values in this report are those found in the actual product's dry or liquid form. In addition, in order to compare concentrations on a solids basis for dried egg products versus corresponding liquid products, concentrations were calculated as 100 g dry weight, using this equation:

[weight of nutrient per 100 g)/(100 g – assayed g moisture per 100 g)] * 100

To conduct statistical comparison of each nutrient mean for the dried *versus* liquid type per part on a dry weight basis, each set of values was modeled separately by nutrient with R, a programming language and software environment for statistical computing (R Core Team, 2018) using linear mixed models (Bates et al., 2015), with the aim of estimating the mean and standard error of the nutrient content based on the egg type (dried, frozen liquid) and part (white, whole, yolk) after accounting for random effects like difference in processors and their drying methods. Appropriate data transforms were used and are described in detail later. Four improbable observations were removed prior to modeling, as noted in Table 4, to satisfy the assumptions of linear mixed models, such as the explanatory variables being linearly related to the dependent variable, normality of residuals and homogeneity of variances across egg parts, types, and processors. The results reported for least squares means were calculated from the respective linear mixed model for each nutrient after taking into account the covariance among different processor, part and type. Hence, means reported here (units/100 grams) may differ from means reported in FoodData Central.

For the statistical analyses, within-processor variances for all three micronutrients were assumed to be homogeneous since the data were not sufficiently large to model heterogeneity in variances across producers without the risk of modeling the noise, such as a faulty lot or a lot consisting of eggs from a relatively small number of hens that were fed a different feed than others under the same processor. Similarly, levels within type (dried, frozen liquid) and part (whole, yolk) for vitamin D_3 and 25(OH)D_3 were also assumed to have similar variances since the data did not require a heterogeneous variance model. For iodine, however, egg white had a relatively low variance compared to the other two parts, hence the iodine model had a heterogeneous variance structure stratified by part.

The models were fitted using restricted maximum likelihood method (REML), and the model having the lowest Akaike Information Criteria (AIC) score was selected for each nutrient (Akaike, 1985). The effect of *Type* (dried, frozen liquid), *Part* (white, whole, yolk) and *Type: Part* interaction, based on the optimum model for each nutrient, was tested for significance using analysis of variance (ANOVA), as shown in Table 5. Least squares mean, standard errors and degrees of freedom [approximated using Kenward-Roger's method (Kenward & Roger, 1997)] were estimated for each nutrient from the nutrient's respective linear mixed model using emmeans package (Lenth, 2018). In cases where the data had been transformed for the analysis, delta method from emmeans package (Lenth, 2018) was used to back-transform the means and standard errors and confidence intervals to the original scales. Note that in such cases the confidence intervals would be asymmetric around the mean. Using the null hypothesis that nutrient contents in frozen liquid eggs are not different from those of the dried form of the same egg part for the processor, pairwise statistical comparison (post-hoc tests) for *Type-Part* combination for each nutrient model was done using emmeans (Lenth, 2018) package. The *p*-

values of interest (dried *vs*. frozen liquid for each part) across all nutrients (total of 6 comparisons) were then adjusted for multiple pairwise comparison using the Hommel correction (Hommel, 1988).

The dependent variable for each of the models was the adjusted dry weight of the respective nutrient, transformed wherever deemed necessary (Table 4).

3. Results and Discussion

Samples received had no added salt or sugar or other ingredients, according to the products' labels and processors' information. Dried egg white samples were described as 'stabilized', as is the typical industry practice of removing glucose before drying for optimized storage (Belyavin, 2003).

3.1. Quality control data

Results for the certified reference materials and in-house control materials are presented in Table 2, indicating that values for certified materials were within previously specified acceptable ranges.

3.2. Nutrient data for processed eggs (per 100 g)

Three analytical samples per processor per category were analyzed, in most cases. For vitamin D_3, 25(OH)D_3, and iodine, sample means and standard deviations (SD) were determined per processor for each egg category. Least squared means and standard errors (SE) for each category were also determined for vitamin D_3, 25(OH)D_3, iodine, and moisture.

3.2.1. Moisture

Moisture levels (g/100 g; mean ± SE) (Table 3) for frozen liquid parts were 56.1 ± 0.2 (yolk), 75.4 ± 0.2 (whole), and 88.4 ± 0.2 (white). Percent solids (the remainder after subtracting percent moisture weight from 100), corresponded closely to industry standard levels of solids reported by Wu (2014), which are 43–44% for yolk, 24.2% for whole, and ~12% for white. Mean moisture for dried parts (g/100 g) were 3.6 ± 0.2 (yolk), 4.1 ± 0.2 (whole), and 7.9 ± 0.2 (white). Moisture for dried egg white in this study corresponded favorably with the industry standard, which is 6.5–8.5%.

3.2.2. Vitamin D_3

Vitamin D_2 was not detected (< 0.1 µg/100 g) in any samples. As expected, vitamin D_3 and 25(OH)D_3 concentrations (µg/100 g) were below the limit of detection (0.1 µg/100 g) in whites of frozen and dried types. For whole eggs, vitamin D_3 (µg/100 g; mean ± SE) on an as-received basis was 1.9 ± 0.5 (frozen liquid) and 7.6 ± 1.8 (dried), and in yolk was 4.8 ± 1.2 (frozen liquid) and 12.2 ± 2.9 (dried) (Table 3). The wide range of concentrations for whole egg and yolk categories among processors can be observed in Figure 1.

The mean concentration (µg/100 g; mean±SE) of 25(OH)D_3 in yolk and whole egg on an as-received basis was, as expected, highest in dried yolk (2.6 ± 0.2) and lowest in frozen whole egg (0.6 ± 0.1). However, within each type (dried or frozen), vitamin D_3 and 25(OH)D_3 concentrations varied widely among individual samples. In fact, some whole egg samples had higher vitamin D levels than some yolk samples (Figures 2, 3, and 4), which was not expected.

A strong correlation between vitamin D_3 and 25(OH)D_3 exists for dried whole (r = 0.791, p <0.001), frozen liquid whole (r = 0.843, p <0.001), dried yolk (r = 0.601, p = 0.01) and frozen liquid yolk (r = 0.624, p = 0.01). To test the correlation between Vitamin D_3 and 25(OH)D_3, values were transformed to log scale so that both nutrients would have similar variance. These correlations are not entirely surprising, since 25(OH)D_3 is a biologically active metabolite of vitamin D_3 and both are typically found in animals (Roseland et al., 2017; Taylor et al., 2014).

For context, dried whole egg values from this study can relate to other sources as points of reference. For example, the National Institute for Standards and Technology (NIST) provides a whole egg powder produced by a commercial manufacturer (NIST, 2019) for use as a standard reference material, and the same material was measured for vitamin D by five labs in an interlaboratory study (Roseland et al., 2015). The means (μg/100 g) for vitamin D_3 and 25(OH)D_3 in this study (7.6 and 1.6, respectively) were slightly higher than the NIST certified range of 4.41–5.35 for vitamin D_3 and 1.07–1.37 for 25(OH)D_3 and were slightly higher than the interlaboratory means (4.49 and 1.25, respectively) which fell within the certified range (Table 2). Nonetheless, since these study results include samples from six processors, it is reasonable that the study's mean would differ to some extent from the certified value of a material sourced from a sole processor.

These results can be considered in context with U.S. dietary intake recommendations using Recommended Dietary Allowances (RDA), which are guidelines indicating average daily intake amounts that meet nutrient requirements of most healthy individuals (Institute of Medicine, 2010). The RDA for vitamin D is 15 μg for persons aged 1–70 years old. As an illustration, 100 grams of frozen liquid whole eggs (equivalent to 2 large eggs) are a reasonable example of a serving size for processed eggs, such as scrambled eggs in a foodservice setting. The estimated contribution of vitamin D_3 from frozen liquid whole eggs using selected values from this study could vary considerably, since the mean per processor ranged from 1.3 to 4.5 μg/100 g, thus would provide as little as 6.5% or as much as 22.5% of the RDA. However, based on the overall mean from this study, a 100-g serving of frozen liquid eggs can provide over 15% of the RDA for vitamin D_3 on average.

3.2.3. Iodine

Among liquid egg products, iodine (µg/100 g) (Table 3) had mean ± SE levels of 172 ± 20 for yolk, 70.6 ± 12.2 for whole, and were below detection for white. In dried forms, mean iodine levels (µg/100 g; mean ± SE) were 339 ± 28 in yolk, 269 ± 23 in whole, and 33 ± 4.7 in white. Variability among samples per processor was relatively low for dried white and frozen liquid whole, but notable variability was observed for dried yolk, frozen liquid yolk, and dried whole from several processors (Figure 5). Further, overlaps were seen between values for frozen liquid yolk and dried yolk (Figure 6).

Considering the results from this study, a serving of frozen liquid eggs (100 g) can provide over 40% of the RDA for iodine on average, based on the RDA of 150 µg for adults ages 18 and older (Institute of Medicine, 2001). However, the estimated contribution could range from 31 to 54% of the DV, depending upon processor, which reflects the variability of iodine concentration. Iodine content in eggs is known to vary according to the amount of iodine consumed by hens in their feed rations (Travnicek et al., 2006), but since most processed eggs are typically used as ingredients in small quantities (e.g., dried egg in baked goods), neither the variability aspect nor quantity consumed would be likely to have a measurable effect on overall nutrient intake.

3.3. Comparison of nutrient concentrations in frozen liquid and dried forms of egg products (per 100 g dry weight)

After adjusting for moisture content, mean concentrations of vitamin D_3, 25(OH)D_3, and iodine per processor were determined, as well as overall means per category, in order to compare dried and frozen liquid counterparts by dry weight. The nutrient mean (per 100 g dry weight) per processor for each egg category, as well as the range of individual values, are plotted in Figure 7 using circles for frozen liquid parts and triangles for dried parts.

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When overall means were compared for dried *versus* frozen liquid counterparts by dry weight, none of the comparisons were significantly different (Table 6) after adjusting for multiple familywise comparisons, as discussed below.

3.3.1. *Vitamin D_3 and 25(OH)D_3 in frozen liquid versus dried eggs (per 100 g dry weight)*

Data for whites were not compared since levels of vitamin D_3 and 25(OH)D_3 in whites were below the limit of detection.

For vitamin D_3, no significant difference (<0.05) was observed between overall means for dried and liquid types for whole or yolk parts in dry weights (Table 6). Similarly, for 25(OH)D_3 no differences between dried and liquid types for whole or for yolk were found (Table 6).

In this study, processors' dehydrating methods appeared to be quite dissimilar from one another, judging by the results, such as a considerable increase in Vitamin D_3 in dried yolk from Processor V (Figure 7). Dried eggs can be produced from spray drying, pan drying, freeze drying, or belt drying methods (Bergquist, 1995; Wu, 2014). Since parameters at processing facilities can differ, such as method, type of equipment, time, and temperature used for dehydrating eggs (Bergquist, 1995), any of those factors could potentially contribute to the variation.

These results showing a range of values among processors for vitamin D_3 and 25(OH)D_3 may be due to varied agricultural practices or differences in the hens' diets such as intentional supplementation of vitamin D_3 or 25(OH)D_3 in the feed which affect egg nutrient content (Browning & Cowieson, 2014; Kuhn et al., 2014: Mattila, 2015; Persia et al., 2013; Surai & Sparks, 2001), as previously mentioned.

3.3.2. *Iodine in frozen liquid versus dried forms of eggs (per 100 g dry weight)*

No significant difference was seen between means for dried and frozen liquid types for yolk, or for whole, by dry weights (Table 6). Frozen liquid and dried whites were not compared, because iodine levels in frozen liquid whites were below detection limits.

3.3.3. *Factors contributing to nutrient variability*

Among factors affecting nutrient variability, the biggest influence on variance in 25(OH)D_3 (~50%) can be explained by *Part* (whole, yolk), since the concentration of 25(OH)D_3 in whole and yolk was much more dissimilar than for vitamin D_3 in this report. The major source of variability for vitamin D_3 was the processors. That is, processors were fairly dissimilar in their products' levels of vitamin D_3 content, and *Processor* was a higher influence on variability for vitamin D_3 as compared to 25(OH)D_3 or iodine. *Processor: Part* interaction was almost negligible across the nutrients. For iodine, since the white part had a relatively small nutrient concentration, the parts were very dissimilar, so almost 75% of the variance could be explained by the difference in parts. *Processor: Type* interaction was higher in vitamin D_3 than the other two nutrients, suggesting that drying processes were likely quite dissimilar among processors, affecting vitamin D_3 more than the other nutrients.

A range of factors impacting variability have been reported in the scientific literature. Enhancing diets of laying hens with iodine, vitamin D, and 25(OH)D can produce eggs with higher levels of these and other micronutrients (Mattila, 2015; Schiavone & Barroeta, 2011; Travnicek et al., 2006). Besides different feed formulas, factors directly influencing nutrient concentrations of eggs include hen genetics, environmental temperature, and egg storage practices (Watkins, 1995). Furthermore, housing systems have been shown to affect levels of vitamin D in eggs, with mixed results. For example, one study found that eggs from free-range hens (exposed to sun) had higher vitamin D and 25(OH)D content than eggs from hens reared in barns (indoors) (Kuhn et al., 2014), while another study found free-range eggs with slightly lower vitamin D than barn eggs (Matt et al., 2009).

3.4. *Limitations*

The means determined for vitamin D and iodine in this study are applicable to the processed egg supply at one point in time, and therefore may be dissimilar to means determined from samples proportional to the entire market. Since the industry partners who provided the samples in this study considered market share information proprietary, it could not be considered in the analysis; thus, processors were not statistically compared. Follow-up investigations using a statistically-based sampling plan in multiple seasons could provide additional breadth for further characterizing the content and variability of vitamin D and iodine over a span of time.

4. Conclusion

Overall mean vitamin D_3, 25(OH)D_3 and iodine content for dried whole egg and yolk compared to their frozen liquid counterparts on a dry weight basis were not significantly different. Updated nutrient data profiles for these processed egg products are accessible for researchers and the general public in FoodData Central, USDA's food composition database (USDA, 2019b), including mean, standard error, median, and individual sample information for each egg category. These data results demonstrate the importance of reporting analytical nutrient values for processed eggs in order to examine factors of variability. Further research will be beneficial to investigate the influence of season, agricultural practices (e.g., hen diet and housing), and other factors on the content and variability of vitamin D and iodine among parts, types, and processors of eggs that could impact estimates of dietary intake.

Author Statement

Janet Roseland: Conceptualization; Methodology; Validation; Investigation; Writing-Original draft; Writing-Review and editing; Visualization; **Meena Somanchi:** Conceptualization; Methodology; Writing- Review and editing; Funding acquisition**; Rahul Bahadur:** Methodology, Software, Validation; Visualization; Writing-Review and editing; **David Haytowitz:** Conceptualization; Methodology; Writing-Review and editing; Project administration; Funding acquisition; **Pamela Pehrsson:** Conceptualization; Methodology; Writing-Review and editing; Funding acquisition.

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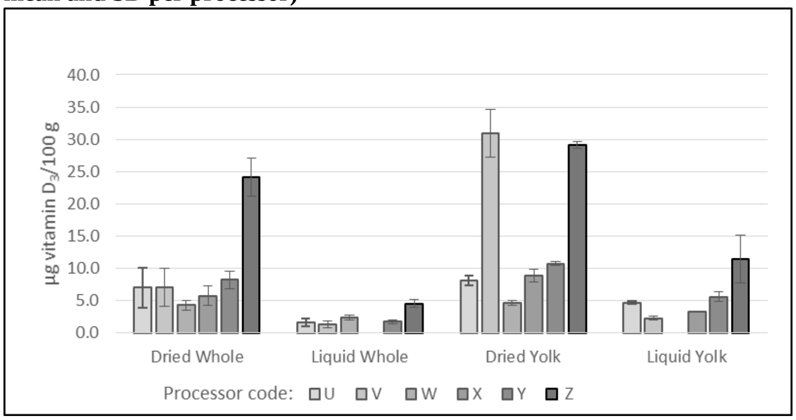
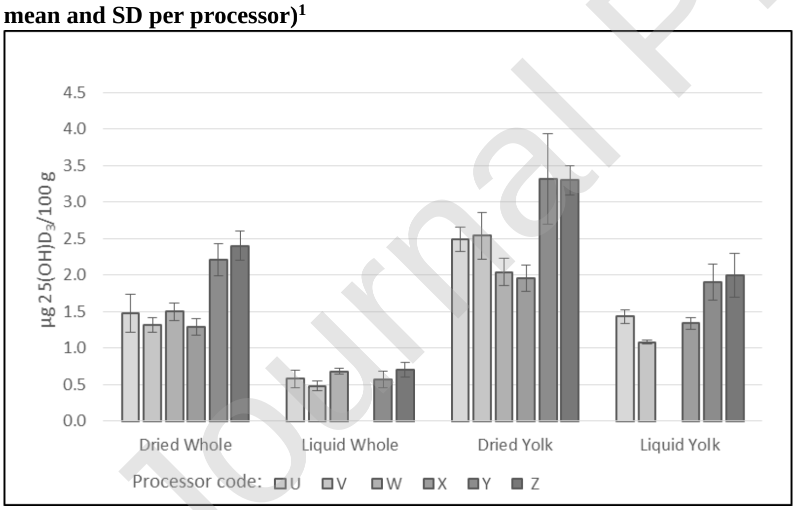
Figure 1. Vitamin D_3 in dried and liquid whole egg and yolk (µg/100 g; mean and SD per processor)[1]



[1]Vitamin D_3 was not detectable for dried or liquid egg white; Processor code is a random letter U through Z.

Figure 2. 25(OH)D_3 in dried and liquid whole egg and yolk (µg/100 g; mean and SD per processor)[1]



[1]25(OH)D_3 was not detectable for dried or liquid egg white; Processor code is a random letter U through Z

Figure 3. Vitamin D_3 and 25(OH)D_3 in dried whole egg and yolk (µg/100 g)

Figure 4. Vitamin D_3 and 25(OH)D_3 (µg/100 g) in liquid whole egg and yolk (µg/100g)

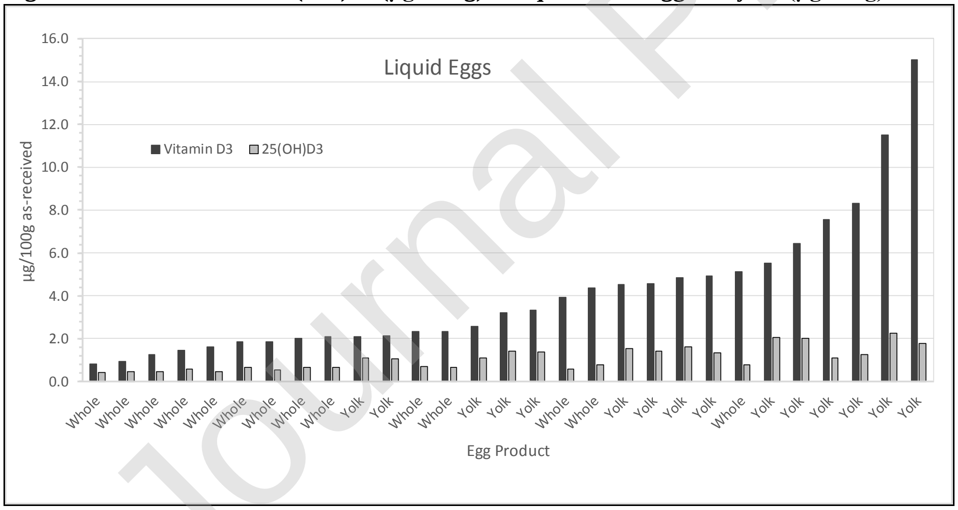
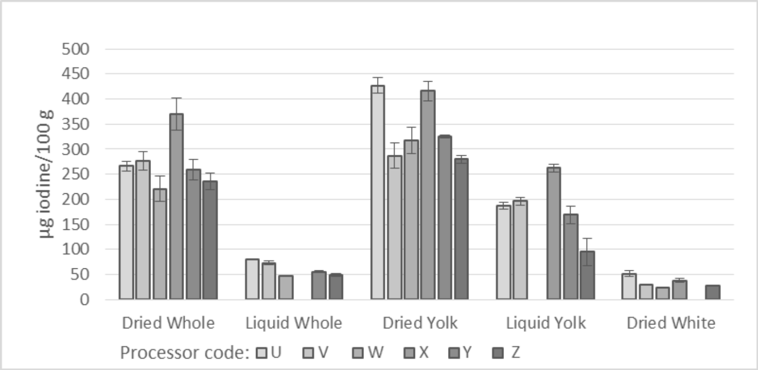


Figure 5. Iodine in dried white, dried and liquid whole egg and yolk (µg/100 g; mean and SD per processor)[1]

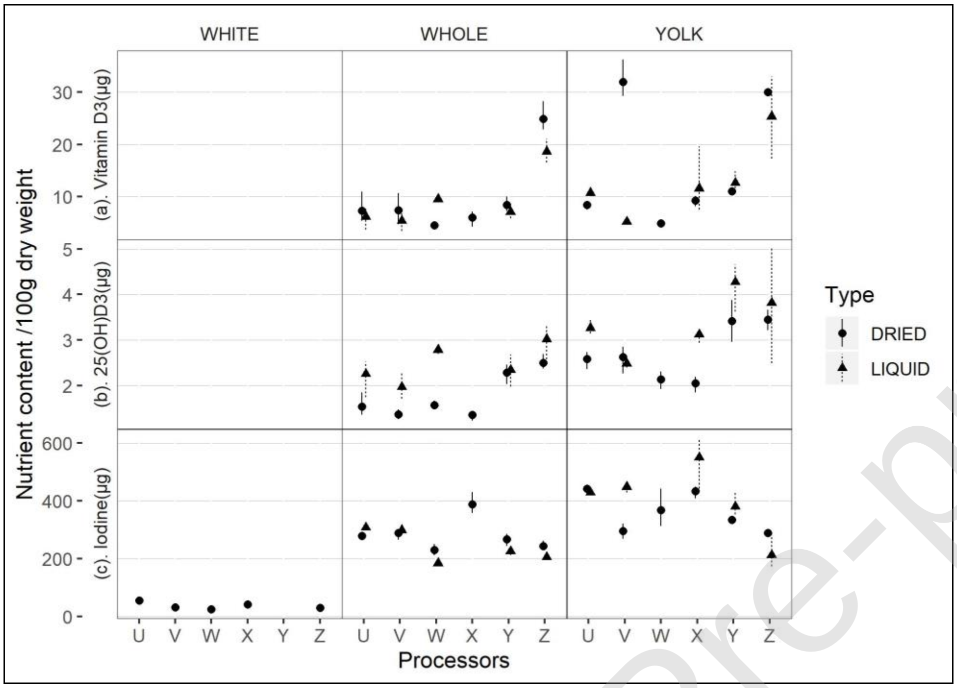


[1]Iodine was not detectable for dried or liquid egg white; Processor code is a random letter U through Z.

Figure 6. Iodine in dried and liquid whole egg and yolk (µg/100g)[1]

[1]Processor code is a random letter U through Z.

Figure 7. Nutrient mean and range for dried white, dried and liquid whole egg and yolk, per processor (ug/100 g dry weight)[1]



[1]Plotted symbols depict mean per processor-part-type combination (after calculations which converted original weights to dry weights). Vertical lines around each symbol represent range of individual values.

Table 1. Laboratory methods used for analyzing nutrient components in dried and frozen liquid whole egg, yolk, and white

Nutrient components	Description	Analytical method
Moisture	Vacuum oven at 100 ℃; ~2 g samples for dried; ~5 g samples for frozen liquid	AOAC 926.08 (33.7.03) (AOAC, 2017)
Vitamins D_3, 25(OH)D_3, and D_2	HPLC–MS/MS; (D_3 [$^2H_3$] internal standard); ~2–3 g samples for dried; ~4–8 g samples for frozen liquid	Huang, LaLuzerne, Winters, Sullivan. (2009). *JAOAC Int.*, 92, 1327-35
Iodine	Inductively coupled plasma mass spectrometry (ICP–MS); ~ 1 g samples	Sullivan, Zywicki. (2012). *JAOAC Int.*, 95, 195-202

Table 2: Expected ranges and results for laboratory quality control materials used in the analysis of nutrient components in dried and frozen liquid whole egg, yolk, and white

			Assayed with [b]						Assayed				Expected	
Component	**Units**	**Material[a]**	**DE**	**DY**	**DW**	**LE**	**LY**	**LW**	***n***	**Mean**	**%RSD**	**HorRat[c]**	**Range**	**Source of expected[d]**
Vitamin D_3	µg/100g	NIST SRM® 1845a Whole Egg Powdera	X	X		X	X		3	4.28	2.5	0.2	4.41–5.35	COA (certified)[e]
		Vitamin D Fortified Cheese Control Composite[f]				X	X		1	7.94			6.20–8.90	Phillips et al. (2008)[g]
		Whole Egg Control Composite[f]				X	X		1	1.67				no data
		Egg White Control Composite[f]			X			X	1	**<0.1**				no data
25(OH) D_3	µg/100g	NIST SRM® 1845a Whole Egg Powder[a]	X	X		X	X		3	0.91	5.4	0.3	1.07–1.37	COA (certified)[e]
		Vitamin D Fortified Cheese Control Composite[f]				X	X		1	0.06				no data
		Whole Egg Control Composite[f]				X	X		1	0.54				no data
		Egg White Control Composite[f]			X			X	1	0.03				no data
Iodine	µg/100g	NIST SRM® 1849a Adult/Infant Nutritional Formula[a]	X	X	X	X	X	X	1	128			118–140	COA (certified)[e]
		Whole Egg Control Composite[f]	X	X	X	X	X	X	5	79.4	2.2	0.3		no data
		Mixed Dish V Control Composite[f]	X	X	X	X	X	X	1	**<0.1**				no data
Moisture	g/100g	Flour Control Composite[f]	X	X	X				3	12.9	1.6	1.2	11.3–13.1	In-house limits
		Whole Egg Control Composite[f]				X	X	X	4	76.3	0.3	0.3		no data
		Egg White Control Composite[f]				X	X	X	3	88.1	0.1	0.1		no data
		Beef Baby Food Control Composite[f]				X	X	X	1	82.9			82.7–83.3	in-house limits
		Mixed Dish V Control Composite[f]				X	X	X	1	71.7			71.4–71.9	in-house limits

[a]NIST SRM=National Institute for Standards and Technology Standard Reference Material

[b] L= Liquid, D= Dry, E= whole eggs, Y= yolks, W= egg whites

[c]Assayed RSD/expected RSD calculated according to Horwitz, W.; Albert, R. (2006).

[d]COA = certificate of analysis; In-house- Established limits (mean ± 2SD); no data= no previous values (results given for reference)

[e]National Institute for Standards and Technology. (2019). Certificate of Analysis available at: https://www.nist.gov/srm

[f]CC=Control composite developed for the National Food and Nutrient Analysis Program (NFNAP) according to Phillips, K.M., et al. (2006).

[g]Phillips, K.M., et al. (2008).											

Table 3: Nutrient mean and standard error of the mean (SEM) for five nutrient components, per processed egg category (units/100 g as assayed)

Nutrient	Type	Part	N	Unit (per 100g as assayed)	LS Mean[A]	SEM	Degrees of Freedom	Lower CL[B]	Upper CL[B]
Vitamin D_3	Dried	Whole	17	µg	7.6	1.8	9	4.4	13.0
Vitamin D_3	Liquid	Whole	14	µg	1.9	0.5	10	1.1	3.2
Vitamin D_3	Dried	Yolk	17	µg	12.2	2.9	9	7.1	21.0
Vitamin D_3	Liquid	Yolk	14	µg	4.8	1.2	10	2.8	8.2
Vitamin D_3	Dried	White	15	µg	ND	–	–	–	–
Vitamin D_3	Liquid	White	16	µg	ND	–	–	–	–
25(OH)D_3	Dried	Whole	17	µg	1.6	0.2	8	1.3	2.0
25(OH)D_3	Liquid	Whole	14	µg	0.6	0.1	9	0.5	0.7
25(OH)D_3	Dried	Yolk	17	µg	2.6	0.2	8	2.1	3.2
25(OH)D_3	Liquid	Yolk	14	µg	1.5	0.1	8	1.2	1.9
25(OH)D_3	Dried	White	15	µg	ND	–	–	–	–
25(OH)D_3	Liquid	White	16	µg	ND	–	–	–	–
Iodine	Dried	Whole	17	µg	269	23.4	6	215	330
Iodine	Liquid	Whole	14	µg	70.6	12.2	6	44	104
Iodine	Dried	Yolk	16	µg	339	28	6	274	412
Iodine	Liquid	Yolk	14	µg	172	20	6	126	224
Iodine	Dried	White	15	µg	33	4.7	6	22.7	46
Iodine	Liquid	White	16	µg	ND	–	–	–	–
Moisture	Dried	Whole	17	g	4.1	0.2	21	3.6	4.5
Moisture	Liquid	Whole	14	g	75.4	0.2	27	75.0	75.9
Moisture	Dried	Yolk	17	g	3.6	0.2	21	3.2	4.1
Moisture	Liquid	Yolk	15	g	56.1	0.2	25	55.7	56.6
Moisture	Dried	White	15	g	7.9	0.2	25	7.5	8.4
Moisture	Liquid	White	16	g	88.4	0.2	23	87.8	88.8

[A]ND = not detectable
[B]CL = 95% confidence limit

Table 4. Models used for statistical analyses of three micronutrients in dried and frozen liquid whole egg, yolk, and white

Nutrient	Dependent variable	Fixed effect	Random effect	Observation removed
Vitamin D_3	Log(Vitamin D_3)	Type * Part	(1 | processor) + (1 | processor:Type)	56th Assayed value = 8.32 (liquid yolk)
25(OH)D_3	Log(25OHD_3)	Type + Part	(1 | processor) + (1 | processor:Type)	62nd Assayed value = 1.09 (liquid yolk)
Iodine	Square Root(Iodine)	Type * Part	(0+ Part | processor) + (1| processor:Type),	1st, 28th Assayed values = 423; 188 (dried yolk, liquid yolk)

Table 5. Analysis of variance statistics for three micronutrients in dried and frozen liquid whole egg, yolk, and white (Type II Wald F-tests with Kenward-Roger df)

Nutrient	Term	***F***[A]	**Df**[B]	**Df.res**[C]	***p*-value**[D]
Vitamin D_3	Type[E]	0.14	1	4.959	0.7226
	Part[F]	26.87	1	49.034	<0.001*
	Type:Part[G]	0.54	1	49.241	0.4681
25(OH)D_3	Type	15.82	1	4.946	0.01079*
	Part	156.55	1	50.813	<0.001*
Iodine	Type	0.25	1	5.052	0.6361
	Part	218.47	2	3.848	<0.001*
	Type:Part	4.77	1	58.21	<0.05*

[A]*F* statistics for the term.
[B]Numerator degrees of freedom.
[C]Denominator degrees of freedom.
[D]*Denotes significant *p*-value
[E]Type is factor with 2 levels (Dried, Liquid).
[F]Part is factor with 2 levels for vitamin D_3 and 25(OH)D_3, and 3 levels for iodine (White, Whole, Yolk).
[G]Type:Part is the interaction between Type and Part.

Table 6: Nutrient mean and standard error of the mean (SEM) for three micronutrients, per processed egg category (units/100 g dry weight)

Nutrient	Type	Part	N	Unit (per 100 g dry weight)	LS Means[A]	SEM	Degrees of Freedom	Lower CL[B]	Upper CL[B]
Vitamin D_3	Dried	Whole	17	µg	7.9[a]	1.9	9	4.6	13.5
Vitamin D_3	Liquid	Whole	14	µg	7.6[a]	1.9	10	4.4	13.1
Vitamin D_3	Dried	Yolk	17	µg	12.7[b]	3.0	9	7.4	21.7
Vitamin D_3	Liquid	Yolk	14	µg	10.8[b]	2.6	10	6.3	18.6
Vitamin D_3	Dried	White	15	µg	ND	–	–	–	–
Vitamin D_3	Liquid	White	16	µg	ND	–	–	–	–
25(OH)D_3	Dried	Whole	17	µg	1.7[a]	0.2	8	1.4	2.1
25(OH)D_3	Liquid	Whole	14	µg	2.3[a]	0.2	8	1.9	2.9
25(OH)D_3	Dried	Yolk	17	µg	2.6[b]	0.2	8	2.1	3.2
25(OH)D_3	Liquid	Yolk	14	µg	3.6[b]	0.3	8	2.9	4.4
25(OH)D_3	Dried	White	15	µg	ND	–	–	–	–
25(OH)D_3	Liquid	White	16	µg	ND	–	–	–	–
Iodine	Dried	Whole	17	µg	281[a]	33.6	6	205.8	367
Iodine	Liquid	Whole	14	µg	276[a]	33.8	6	200.9	362
Iodine	Dried	Yolk	16	µg	352[b]	39.5	6	263.2	454
Iodine	Liquid	Yolk	14	µg	387[b]	42.1	6	292.7	494
Iodine	Dried	White	15	µg	36.1	8.6	7	18.7	59
Iodine	Liquid	White	16	µg	ND	–	–	–	–

[A]Paired least squared (LS) means lacking a common superscript within an egg part differ ($p < 0.05$).

[B]CL = 95% confidence limit