# A revolutionary kit measures egg yolk carotenoids

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Carotenoids in feed are not only responsible for yolk colour but are also health promoting antioxidants and a precursor of vitamin A. The colour of yolk can only be predicted and controlled through the control of the carotenoid content in the feed. Controlling the colour is important because consumers in various countries prefer yolks of certain defined and reproducible hues of yellow-orange.

## Visual assessment

The simplest method in assessing yolk colouration is a visual empirical estimation of carotenoids. For example, the DSM Yolk Colour Fan has become the instrument most commonly used worldwide to measure the colour of an egg yolk.

However, this method only gives information on colour, not on the content of the biologically important carotenoids that have health promoting effects. Therefore, different chemical methods have been developed to quantitate either total carotenoids or individual carotenoids in egg. The simplest one is the spectroscopic determination of total carotenoids as equivalents of ß-carotene (AOAC 1973). To determine the concentration of individual carotenoids, however, the carotenoids have to be separated by HPLC prior to analysis.

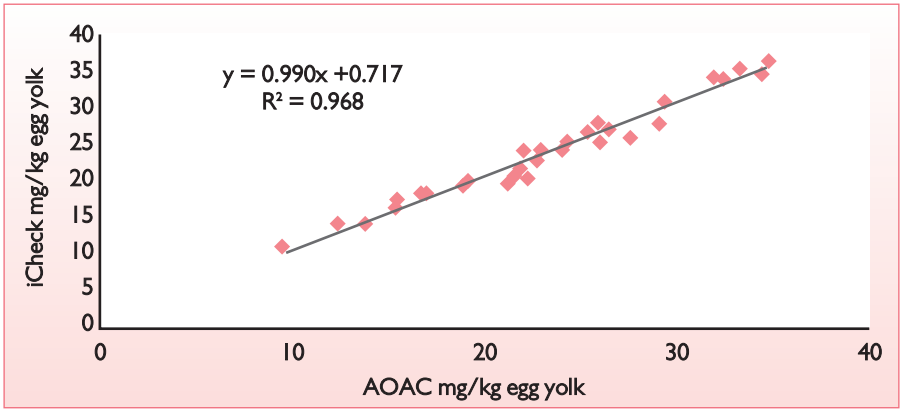
Both methods require a laboratory and sophisticated technical equipment. DSM have introduced a new, fast, easy to perform and laboratory independent assay for the determination of total carotenoids in egg and egg products.

The test consists of two components, a portable spectrophotometer and a disposable analytical unit. The results produced by the new analytical method are compared with those obtained by the AOAC and HPLC methods. To demonstrate the efficacy of this new assay, eggs were bought at random at the local market. Albumen and egg yolk were separated, and the egg yolk was mixed to be separated into equal amounts for comparative analysis between the new method and the AOAC method.

## Comparison with HPLC

For the comparison with HPLC, laying hens were fed in the animal nutrition research centre of DSM with diets containing various concentrations and combinations of apoester (ethyl-ß-apo-8'-carotenoate as Carophyll Yellow 10%), canthaxanthin (as Carophyll Red 10%) and Marigold carotenoids lutein and zeaxanthin. For each treatment four repetitions with 12 hens each were run.

***Fig. 1. Correlation of the total carotenoid results with the new method and with the standard AOAC method.***




● **New method:** The iEx/iCheck method consists of a disposable all inclusive extraction and measuring unit, the iEx, and a battery driven hand-held photometer, the iCheck. With this assay, 400mg of yolk is diluted to a final weight of 2g and 400µl of the diluted yolk is injected into the extraction vial.

Thereafter, it is shaken for 10 seconds and left for complete phase separation for at least five minutes. This extracts all carotenoids present in the sample into the upper organic phase. The concentration is measured in the portable photometer and final concentration is calculated.

● **Spectroscopy:** The AOAC method was performed as follows: to 1g of yolk from one egg, acetone was added in two steps, first 1ml to make a smooth paste and then 50ml. The solution was mixed and filtered. After washing the filter with acetone, the recovered acetone was diluted to 100ml.

Yolk colour equivalent to µg ß-carotene/g sample was measured on a spectrophotometer at 450nm wavelength.

● **HPLC:** The yolk was mixed with water and ethanol and the mixture was extracted with n-hexane. Aliquots of the upper hexane phase were injected into various isocratic normal-phase HPLC systems which used all the same stationary phase but different acetone/n-hexane mixtures as mobile phases.The HPLC systems were able to separate and quantify all-E- and Z-isomers of apoester, canthaxanthin, lutein, and zeaxanthin.

● **Statistical analysis:** The results were analysed by three different methods. Correlation of results obtained were analysed using regression analysis. The degree of agreement between the two methods was evaluated by examination of the Bland-Altman difference plots. Good agreement with no real bias was indicated when the 95% confidence interval for the bias including zero.

● **Comparison with the AOAC method:** Results indicated that there was no significant difference for all egg yolk samples between the two methods. The levels in the AOAC group were slightly higher with correlation (r>0.98) between the two methods being excellent for total carotenoids.

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● **Comparison with HPLC:** From the results it was shown that the iCheck method found higher total carotenoid contents compared to HPLC and that this over estimation depends on the amount of the extracted sample portion. If the yolk contained mainly canthaxanthin besides minor amounts of the yellow carotenoids lutein and zeaxanthin, the iCheck assay led to lower total carotenoid contents compared to HPLC.

This under estimation was expected since canthaxanthin is a reddish pigment with an absorption maximum at 466nm which is not adequately covered by the β-carotene equivalent method using a detection wavelength of 450nm.

This finding is certainly more of academic interest than of practical importance since canthaxanthin and other red carotenoids are usually fed in combination with yellow carotenoids in order to obtain an acceptable yolk colour. In yolks with various combinations of canthaxanthin and apoester the iCheck analyses of the egg yolks agreed within 12% with the HPLC results.

In accordance with the finding above, carotenoid mixtures with higher canthaxanthin concentrations and lower contribution of yellow carotenoid tended to be under estimated, whereas those with higher apoester concentrations were rather over estimated by the iCheck assay.

## Conclusion

The comparison of the new iCheck assay to analyse total carotenoids in egg yolk showed an excellent agreement with the standard AOAC method. This is expected as both methods use the total absorption of all present egg yolk carotenoids and are based on the calibration with ß-carotene as an external standard. However, the iCheck results also agreed (within 15%) with the total carotenoid contents measured by HPLC in a wide range of carotenoid concentrations and combinations.

This good correspondence is remarkable as the HPLC results derive from a different quantitation principle representing the sum of separated and specifically quantified carotenoids. Comparing the necessary time and technical equipment, the innovative one-step extraction system is superior to the traditional methods with regard to the ease of application and gives results comparable to the standard methods.

The analytical quality is in excellent agreement to the laboratory analyses by the standard AOAC method and in acceptable accordance to HPLC. Besides its ease of performance and analytical sensitivity, the novel system is ecologically superior to previous methods. The miniaturised all-in-one system reduces the exposition of the laboratory personnel to potentially dangerous organic solvents to a minimum, thus reducing environmentally and ecologically critical waste dramatically. ■