

## Ensuring the Reliability of Analytical Data for Carotenoids and Flavonoids: Challenges and Progress

**Delia B. Rodriguez-Amaya**

**Department of Food Science  
Faculty of Food Engineering  
University of Campinas**



### PREMISES

- ☀ **The quality of a database depends on quality of the data generated.**
- ☀ **The quality of the data generated depends on the quality of the sample submitted to analysis.**



## **REQUIREMENTS FOR RELIABLE RESULTS**

- ☀ **Representative sampling**
- ☀ **Validated method**
- ☀ **Quality assurance system**
- ☀ **Adequately trained analyst**



---

---

---

---

## **Determination of bioactive compounds is a challenging task because:**

- ☀ **They are generally present at very low levels together with other compounds, at much higher concentrations, that may interfere in the analysis.**
- ☀ **There are many classes of compounds now considered to be health promoting, each group consisting of many members with differing biological activities or efficacies, thus requiring separation, identification and quantification of individual compounds.**
- ☀ **Many are unsaturated, susceptible to degradation during analysis, with members of a group capable of degrading at varying degrees.**



## Difficulties in carotenoid analysis are due to:

- ☀ **Marked qualitative and quantitative variation of the carotenoid composition of foods**
- ☀ **Wide concentration range among carotenoids in a given food**
- ☀ **Uneven distribution of carotenoids within a sample and between samples of a given food**
- ☀ **Variation in the nature of the matrix**
- ☀ **Susceptibility of carotenoids to isomerization and oxidation during analysis and during storage of samples and standards.**

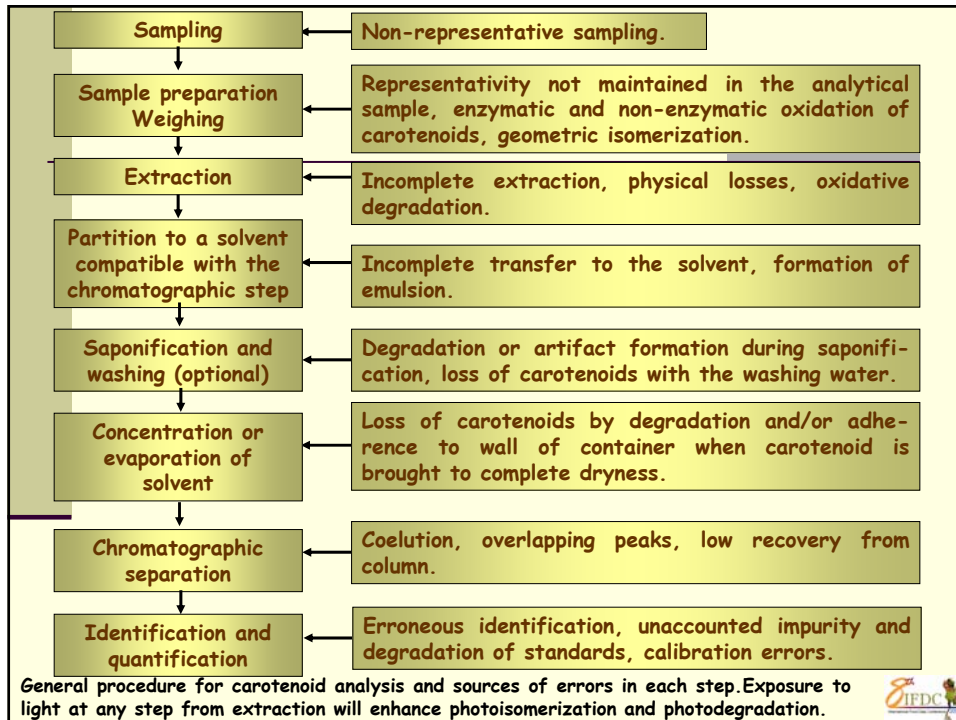
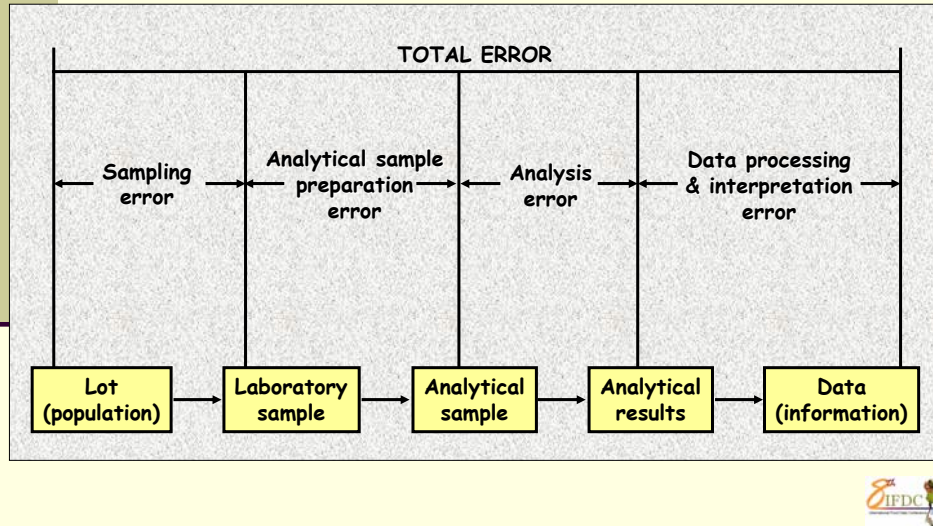


## PROGRESS IN CAROTENOID ANALYSIS

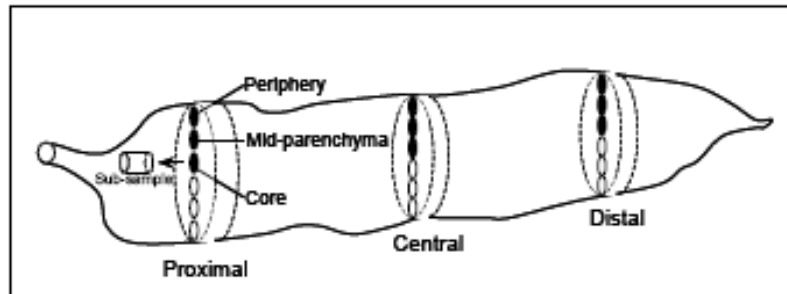
- ☀ **Critical steps and sources of errors have been identified.**
- ☀ **Certified reference materials have been developed.**
- ☀ **Interlaboratory studies have been conducted**



## ILLUSTRATION OF THE TOTAL ERROR IN DATA GENERATION



### Illustration of the sampling procedure of Chavez et al. (2008)



Results: The carotenoid content was higher in the part of the root closest to its attachment to the stem (proximal section), gradually decreasing toward the opposite end (distal section). Across the root, carotenoid content was higher in the core, lower towards the periphery.



### For carotenoids, two certified reference materials have been developed:

- ☀ **The Community Bureau of Reference BCR485 (freeze-dried mixed vegetables).**
- ☀ **NIST SRM 2383 (baby food composite).**



## EUROPEAN INTERLABORATORY STUDY

### Scott *et al.* (1996)

17 European laboratories

Lyophilized vegetable mix used as potential reference material

Carotenoids measured – lutein, zeaxanthin, lycopene,  $\alpha$ -carotene,  $\beta$ -carotene

Results suggested:

The effect of the chromatographic system is probably not a major variable.

In the more experienced laboratory, variation in the standardization of the carotenoid solution was not thought to be a significant problem.

There were greater variations for lycopene calibration and measurement

The preparation of the extract may account for about 13% of the overall variance of around 23%.



## EUROPEAN INTERLABORATORY STUDY

### Schüep and Schierle (1999)

14 European laboratories

Carotenoids determined -  $\beta$ -carotene and its Z-isomers

4 commercial products – margarine, vitamin drink, pudding powder, natural mixed vegetables

The supplemented drink showed the best repeatability while the pudding powder gave the worst results.

Excluding the Z-isomers from quantification of  $\beta$ -carotene would result in significant underestimation of  $\beta$ -carotene.



## **NIST INTERLABORATORY STUDY**

### **Sharpless *et al.* (1999)**

**26 US and European laboratories**

**Baby food composite as reference material**

**The relative expanded uncertainties were higher than those generally expected for certified values.**

**Certified concentrations were provided for some carotenoids, but only reference values were obtained for other carotenoids.**

**Only reference values were provided for lycopene (all-*E*- and total) because of its instability.**



## **HARVESTPLUS INTERNATIONAL INTERLABORATORY STUDY**

### **Rodriguez-Amaya *et al.* (unpublished)**

**Test materials – sweetpotato, cassava and maize flours.**

**19 Asian, African, European, Latin American and US laboratories.**

**Widely varying laboratory conditions and experience in carotenoid analysis.**

**Results:**

- 1. Incomplete extraction is a major source of error.**
- 2. HarvestPlus had several laboratories that can determine the carotenoid contents of sweet potato and cassava accurately, but more work had to be done with maize.**
- 3. Training of analysts was very important.**



## Quantitative determination of individual glycosides is difficult because:

- ☀ Several glycosides can occur for each flavonoid.
- ☀ Standards are not commercially available for most of these compounds.



## DETERMINATION OF FLAVONOL

### Critical step – hydrolysis / extraction

**Hertog *et al.* (1992)**

Refluxing for 2 h at 90°C with 1.2 M HCl in 50% aqueous methanol.

**Häkkinen *et al.* (1998)**

Using a mixture of flavonol and phenolic acid standards, best results were obtained with 1.2 M HCl at 90°C for 2 h and 0.6 M HCl at 35°C for 16 h. For blackcurrant and strawberry, the method of choice was hydrolysis at 35°C for 16 h with 1.2 M HCl.

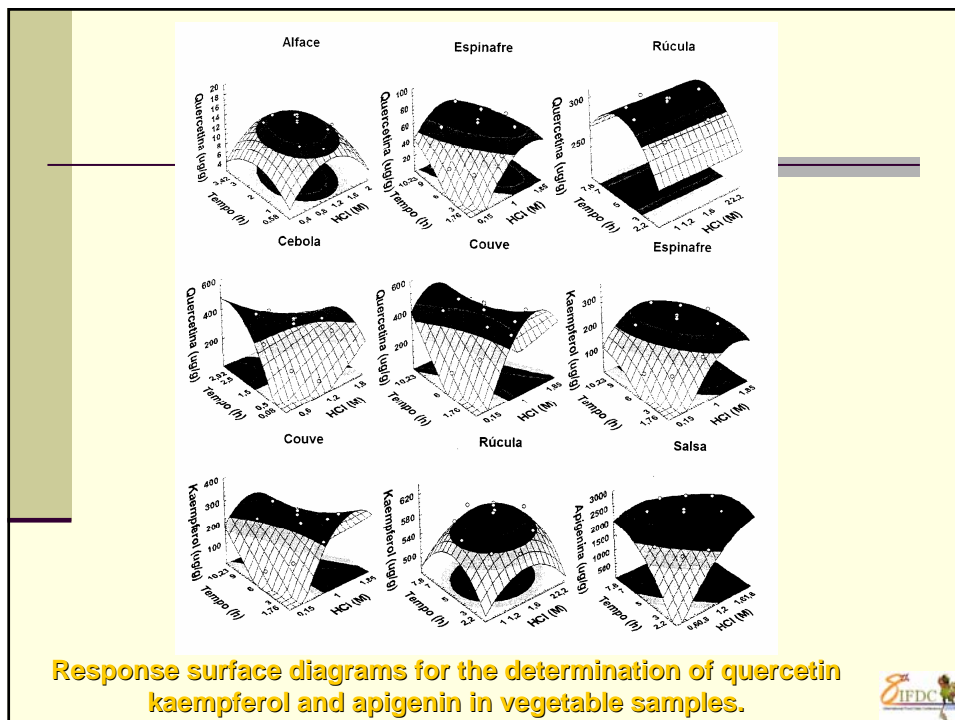


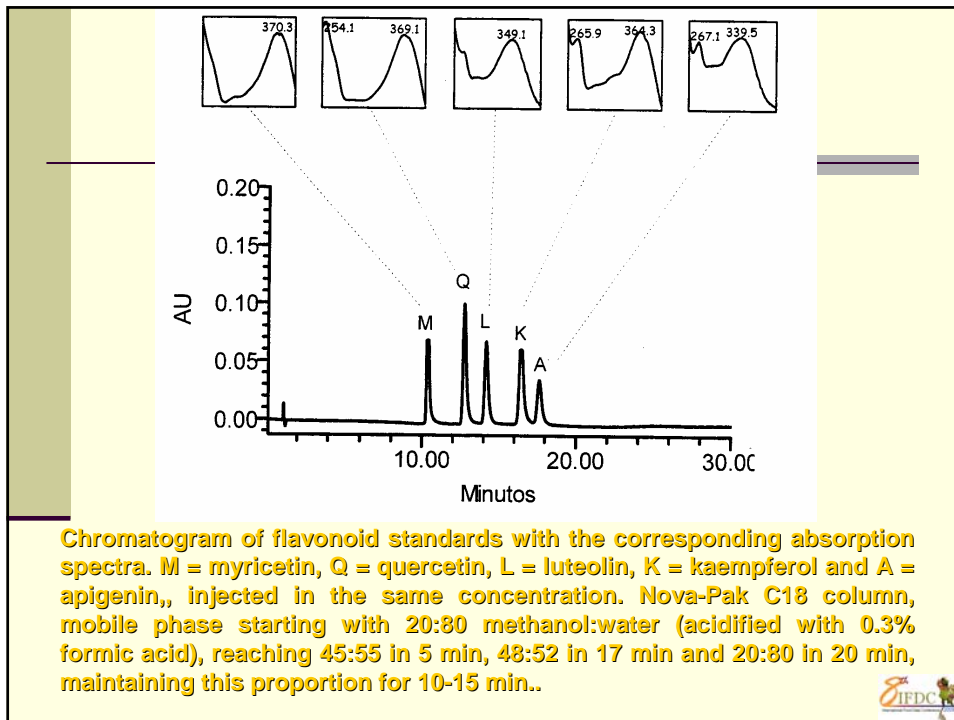
## DETERMINATION OF FLAVONOL

### Critical Step – hydrolysis / extraction

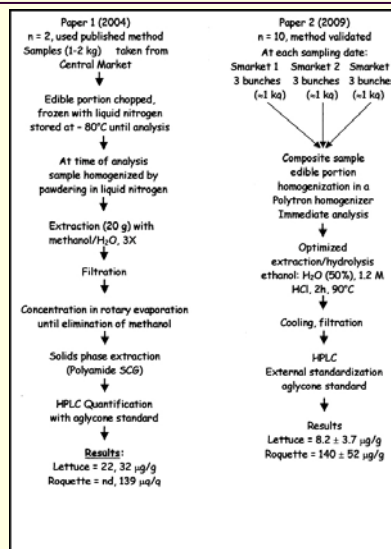
Nuutila *et al.* (2002)

Compared the methods of Hertog *et al.* and Häkkinen *et al.*, using individual standards or mixtures of standards (phenolic acids, flavonols, flavones and catechins). The best results for majority of the compounds were obtained with Hertog *et al.*'s condition.





## DETERMINATION OF QUERCETIN



## PREMISES

- ☀ **The quality of a database depends on quality of the data generated.**
- ☀ **The quality of the data generated depends on the quality of the sample submitted to analysis.**



---

---

---

---

## REQUIREMENTS FOR RELIABLE RESULTS

- ☀ **Representative sampling**
- ☀ **Validated method**
- ☀ **Quality assurance system**
- ☀ **Adequately trained analyst**

