IMPROVEMENT OF A COLORIMETRIC ASSAY FOR THE DETERMINATION OF TOTAL DIETARY POLYPHENOLS IN ORDER TO COMPLETE FOOD COMPOSITION TABLES

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INTRODUCTION

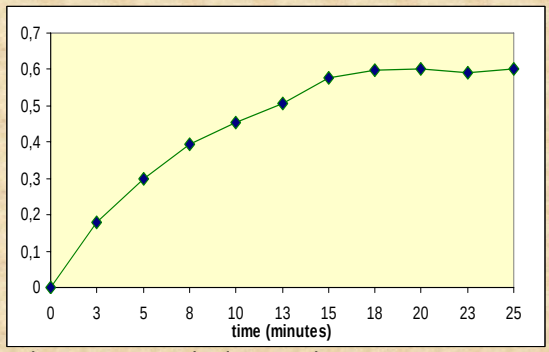
In order to understand the relationships between diet and health, it is necessary to get more data on the composition in protective micronutrients of the most commonly foods and beverages. Polyphenols are the most abundant micronutrients in plant foods. Their estimation remains problematic due to the diversity of structures and the lack of commercial standards, especially for procyanidins. The Folin-Ciocalteu (FC) colorimetric assay, based on the formation of blue complexes between Folin-Ciocalteu reagent and polyphenols, can be used for the quantification of Total Polyphenolic Content. However, FC reagent usually leads to an over-estimation due its reactivity with other non-polyphenolic reducing compounds such as ascorbic acid, reducing sugars, thiols or amino-acids. We report here a method for the elimination of these interfering substances using solid-liquid adsorption technique. We also optimized several parameters of the FC colorimetric assay.

MATERIALS AND METHODS

The solubility of polyphenols in different solvents was tested. The best result was obtained for the mixture water-acetone 30/70 (v/v) .
Folin-Ciocalteu assay consists in two steps : **(1)** the reaction with FC reagent **(2)** the development of blue colored complexes after addition of sodium carbonate. The colored complexes are measured at 760 nm. Gallic acid is usually used as standard.
Different reaction times and temperatures were applied for the two reactions **(1)** and **(2)**.
To eliminate interfering substances, a solid-phase extraction was performed on an Oasis™ HLB cartridge (60 mg packing, Waters, Milford, MA, USA). Packing consists in diyvynil-benzene and pyrrolidone, which have a strong affinity for polyphenols. The acetonic extract was subjected to an Oasis™ HLB cartridge. All the parameters were optimized (quantity, nature and volume of elution solvents) with different polyphenols (gallic acid, ferulic acid, catechin, quercetin, naringin) and interfering substances (ascorbic acid, cysteine).

RESULTS

Evolution of absorbance at 760 nm during 25 minutes of reaction (2) after addition of sodium carbonate Temperature was fixed at 50 °C



Plateau was reached at 15 minutes

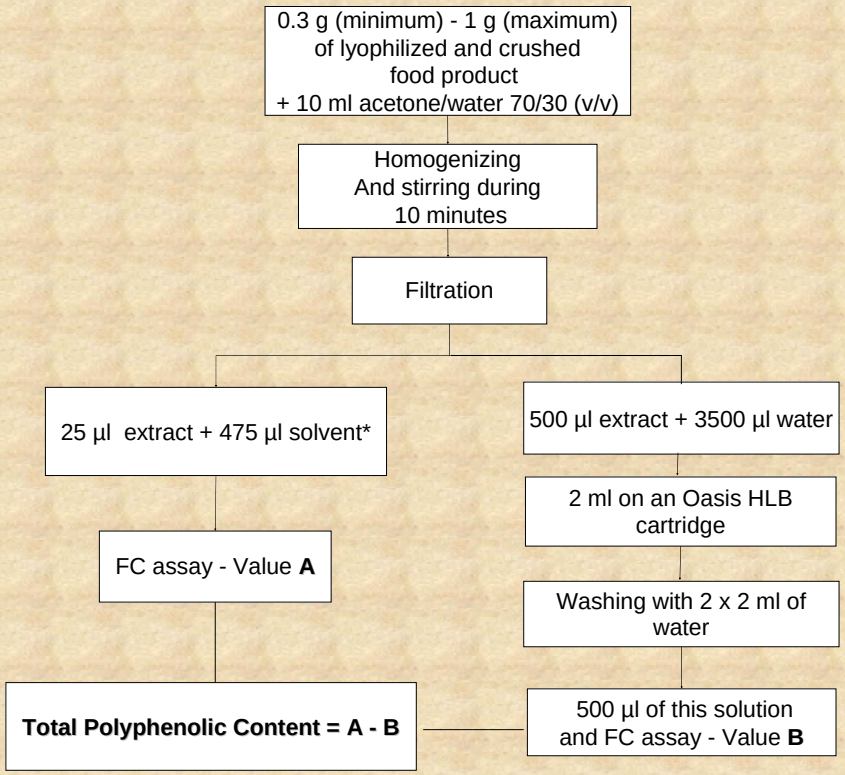
Optimized procedure of FC assay

In a tube, take 25 µl of extract + 475 µl of solvent*
* solvent : water or pure methanol
Add 2.5 ml of FC reagent (1/10 commercial dilution)
Wait for 2 minutes at room temperature
Add 2 ml of sodium carbonate (75g/L)
Keep during 15 minutes at 50°C
Cool rapidly in a bath
Read the absorbance at 760 nm

Performance (% recovered polyphenol) of solid-phase extraction (mean of 4 assays)

	Initial solution	Washing solution	Desorption solution
Gallic acic	100%	-	98%
Ferulic acic	100%	-	93%
Catechin	100%	-	98%
Quercetin	100%	-	95%
Naringin	100%	-	100%
Procyanidin B2	100%	-	65%
Ascobic acid	100%	98%	2%
Cystéine	100%	96%	4%

Final procedure



Water and methanol respectively used for washing and elution steps gave the best results.
Ascorbic acid and other amino acids as cysteine were almost totally eliminated during the washing step.
The glycosides of flavonoids were totally recovered during the elution step with methanol.
Recovering was less for ferulic acid but remained high (93%). For all the procyanidins tested, such as procyanidin B2, desorption step was not efficient (60-65%). For this reason, we concluded that Total Polyphenolic Content could be estimated after subtraction of FC colorimetric value of washing solution.
This procedure was validated with different fruits extracts.

CONCLUSIONS

An improvement of a previously described method using Folin-Ciocalteu reagent (1) was achieved for the determination of Total Polyphenol Content. In order to eliminate interfering substances such as vitamin C, present in high quantities in fruits, the procedure was simplified by employing a solid phase extraction. The method proposed appeared very simple and rapid for the quantitative determination of total polyphenols. Such method could be easily used in plant breeding programs, in food industry and in epidemiology studies.

REFERENCES

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