Optimization of Tannin Extraction from Infant Foods

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The design of experiments (DOE) was used in the development of a laboratory procedure for the extraction of tannins from three infant food types comprising different ingredients of vegetable origin and meat. The diversity of vegetables included in the product formulas required the use of DOE to establish parameters that maximize the recovery of tannins using a central composite rotatable design. Once the experimental results from the DOE were obtained, response surface methodology was used to find the best analytical conditions for samples comprising different ingredients. Sample weight was found to be a critical factor in tannin extraction from foods. Different optimal conditions were obtained for samples including soya in the formula.

Keywords: *Weaning foods; tannins; response surface methodology; optimization*

INTRODUCTION

The quantitation of tannins is a fundamental requirement for carrying out nutritional studies (Reed, 1995). Different tannin extraction conditions have been reported for different types of samples, such as leaves (Makkar and Becker, 1993), cereals (Terrill et al., 1992; Youssef, 1998), legumes (Stanley, 1992; Wang et al., 1998), and oilseeds (Mansour et al., 1993). However, methods for use when samples are mixtures of different ingredients of vegetable origin are less frequently encountered. Traditionally, different factors, such as the solvent system, time, and temperature, have been considered in the extraction of tannins from food samples. In contrast, the sample-to-solvent ratio is less commonly considered as a critical factor, although it has been put forward as a factor that might account for differences in polyphenol concentrations reported for the same species (Constantinides and Fownes, 1994).

The food chemist is constantly performing experiments for numerous purposes; one purpose is to optimize an analytical method for determining a component of a certain type of food, given that the method described for one food type may not be suitable for another (Martínez and Rincón, 1997). The food chemist must, in that case, go on optimizing the analytical method using new analytical conditions but considering that the general goal in any optimization is to ascertain the conditions that produce the best output. The design of experiments is a plan for the deliberate variation of variables to study their effect on certain specific results. For example, to determine optimal conditions, rotatable central composite designs are used, as the result of superimposing a factorial at two levels (2^N) on a star design (2N+1) (Araujo and Brereton, 1996). Response Surface Methodology (RSM) comprises a set of techniques used in the empirical study of relationships between one or more responses and a group of input variables to locate the region of highest response values, where the highest is considered to be the best (Cornell, 1990). The aim of this study was to determine the best conditions of tannin extraction from homogenized infant foods comprising a wide range of vegetables as ingredients, when using the solvent extraction system described by Terrill et al. (1992).

Table 1. Vegetable Content (Percent) of Infant Foods

	infant food[a]		
ingredient	CR	VC	LV
carrot	6.67	13.33	13.27
potato		3.40	6.60
tomato	0.57	0.57	
peas	2.0		5.0
onion	1.0	0.50	3.0
rice	8.0	4.20	
celery	0.67	0.67	
soya			3.0

[a] CR, chicken with rice; VC, veal with carrot; LV, lamb with vegetables.

MATERIALS AND METHODS

Materials. Tannin extractions were performed in duplicate on three homogenized infant foods (also called beikosts or weaning foods) comprising vegetables and meat. Beikost samples (250-g bottles) were manufactured by Hero España S. A. (Alcantarilla, Murcia-Spain). First, meat was cooked and vegetables were blanched. All ingredients for each beikost were mixed and homogenized, and the product was subsequently bottled and heated. Because of the diversity of vegetables included in product formulas (Table 1), the chemical structure of the tannins present in samples was assumed to be very diverse, rendering unsatisfactory for our samples the analytical conditions described in earlier papers for raw and single vegetables.

Methods. In this study, the solvent system described by Terrill et al. (1992) was followed for tannin extraction, but sample weight and solvent volume were optimized as critical factors, as described below. Tannins present in the sample were extracted using 30 mL of extracting solution (acetone/water/diethyl ether 4,7/2,0/3,33, v/v/v) in agitation for 30 min at 24 °C. Extracts obtained were centrifuged at 11.500 rpm for 30 min. Residue was extracted twice more, and supernatants obtained were mixed in a decanting funnel. Phase separation was instantaneous, producing an upper phase—containing acetone and diethyl ether—and a lower phase, containing tannins.

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Table 2. Levels of Factors for Optimization of Assay Conditions for Tannin Extraction

		symbol		levels				
factors	unit	coded	uncoded	−1.414	−1	0	+1	+1.414
weight	g	X_1	ϕ_1	0.17	1.00	3.00	5.00	5.83
volume	mL	X_2	ϕ_2	23.8	30.0	45.0	60.0	66.2

Table 3. Experimental Design and Response Values Obtained under Different Extraction Conditions for Each Infant Food Type[a]

trial	run	X_1	X_2	CR	VC	LV
1	7	0	−1.414	0.166	0.056	0.249
2	3	−1	+1	0.604	0.404	0.335
3	8	0	+1.414	0.155	0.066	0.100
4	1	−1	−1	0.188	0.338	0.210
5	10	0	0	0.121	0.115	0.201
6	4	+1	+1	0.105	0.145	0.079
7	11	0	0	0.222	0.075	0.173
8	12	0	0	0.168	0.082	0.166
9	13	0	0	0.146	0.079	0.171
10	2	+1	−1	0.093	0.089	0.224
11	9	0	0	0.061	0.117	0.198
12	6	+1.414	0	0.051	0.063	0.114
13	5	−1.414	0	0.888	0.493	0.828

[a] CR, chicken with rice; VC, veal with carrot; LV, lamb with vegetables.

Spectrophotometric determination of tannins was performed using the vanillin−HCl method, as described by Stanley (1992), at a wavelength of 500 nm with catechin as standard (Sigma, lot 106H0990).

Experimental Design. For the tannin extraction procedure, a central composite rotatable (CCR) design was followed and RSM used to find a relationship between factors and responses. To use the RSM correctly, a rotatable design with an equal predictability in all directions from the center was used, considering the levels for each factor shown in Table 2. The theoretical aspects and experimental implications of RSM have been described elsewhere (Cochran and Cox, 1965). RSM is currently the most popular optimization technique, probably because of its comprehensive theory, reasonably high efficiency, and simplicity (Arteaga et al., 1994).

The response-variable tannin content, as catechin activity, was assumed to be influenced by two independent variables (sample weight and solvent volume) or factors, ϕ_i ($i = 1, 2$), so that $\xi = f(\phi_1, \phi_2)$, where ξ is the response or total tannins expressed as catechin units/100 g dry material, ϕ_1 is the weight sample expressed as g, and ϕ_2 is the dilution volume expressed as mL. So the unknown function f was assumed to be approximated by a second-degree polynomial equation such as

$$\xi = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 + \epsilon$$

where ξ is the response; b_0 (center point of system), b_i (coefficient of linear effects), b_{ii} (coefficient of quadratic effects), and b_{ij} (coefficient of interactive effects) are the different constant coefficients of the model; X_i are the coded independent variables related to factors ϕ_i, and ϵ is the error of model. In RSM work it is advisable to transform natural variables into coded variables, and these coded variables are usually defined as dimensionless with mean zero and the same spread or standard deviation (Myers and Montgomery, 1995). The Design Expert software (Stat-Ease, Inc., Minneapolis) was used to generate design, regression analysis, and plot obtention.

RESULTS AND DISCUSSION

Experimental results for tannin content under different extraction conditions are shown in Table 3. The regression coefficients (b_{ki}) and their statistical significance are presented in Table 4. Analysis of variance (ANOVA) indicates that the model developed for tannin extraction appeared adequate (Table 4) and accounts for 86.9% (CR samples, Table 1), 94.0% (VC samples) and 72.6% (LV samples) of variability ($R^2 \times 100$), respectively.

Table 4. Regression Coefficients in Terms of Coded Factors for Model Fitting for Response and ANOVA *F* Value Obtained for Each Infant Food Type[a]

food[a]	b_1	b_2	b_{11}	b_{22}	b_{12}	F	p
CR	−0.222[b]	0.051	0.146[b]	−0.008	−0.101	9.34	0.0053
VC	−0.139[b]	0.017	0.111[b]	0.002	−0.002	22.10	0.0004
LV	−0.157[b]	−0.029	0.116[b]	−0.032	−0.067	4.65	0.0315

[a] CR, chicken with rice; VC, veal with carrot; LV, lamb with vegetables. [b] Denotes significant effect on the tannin extraction method at 95% confidence level ($p \leq 0.05$).

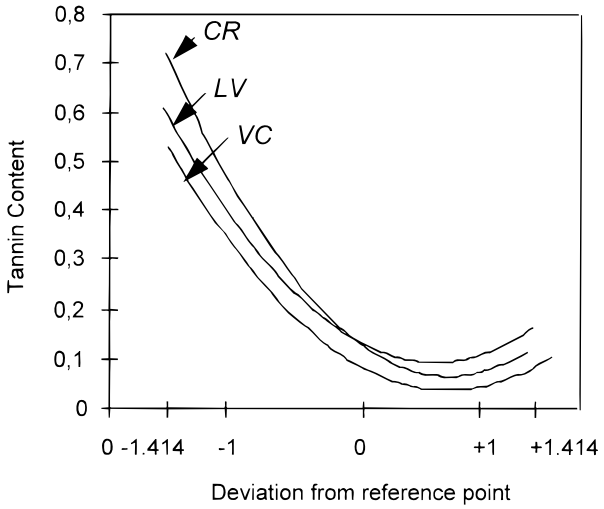

Figure 1. Perturbation plot showing the total effect of sample weight on tannin content extracted from different weaning foods (CR, chicken with rice; VC, veal with carrot; LV, lamb with vegetables).

Considering only the central point replication of the design (Table 3), small range values were observed for VC and LV samples (0.041 and 0.035, respectively), but a very high range value was obtained for CR (0.161); the latter produced a significant lack-of-fit value when the pure error was evaluated in the extraction process of tannins in CR samples. In addition to sample variability due to the range of vegetable ingredients involved, widely varying condensed tannin levels have been reported in aqueous organic solvents, especially acetone; when determinations were performed using the vanillin−HCl method, acetone reacted with acidified vanillin to produce a chromogen with λ_{max} at 548 nm (Makkar and Becker, 1993). Thus, when the botanical origin of the tannins is highly diverse, as in the case of the foods studied, the intensity of this chromogen may vary greatly.

From the ANOVA, the sample weight (W) was shown to be the most critical factor affecting the response through a linear negative effect and a quadratic positive effect for all sample types (Table 4). The simultaneous linear and quadratic effects of the W factor for the three food types are shown in Figure 1. Dilution volume (V) did not have a significant effect on tannin extraction under the experimental conditions studied (Table 2).

Using the procedure described by Cornell (1990), the stationary points were located as $W = 0.17$ g for all sample types and $V = 66.21$ mL for CR and VC types and 45.60 mL for LV type. Graphical location of the best

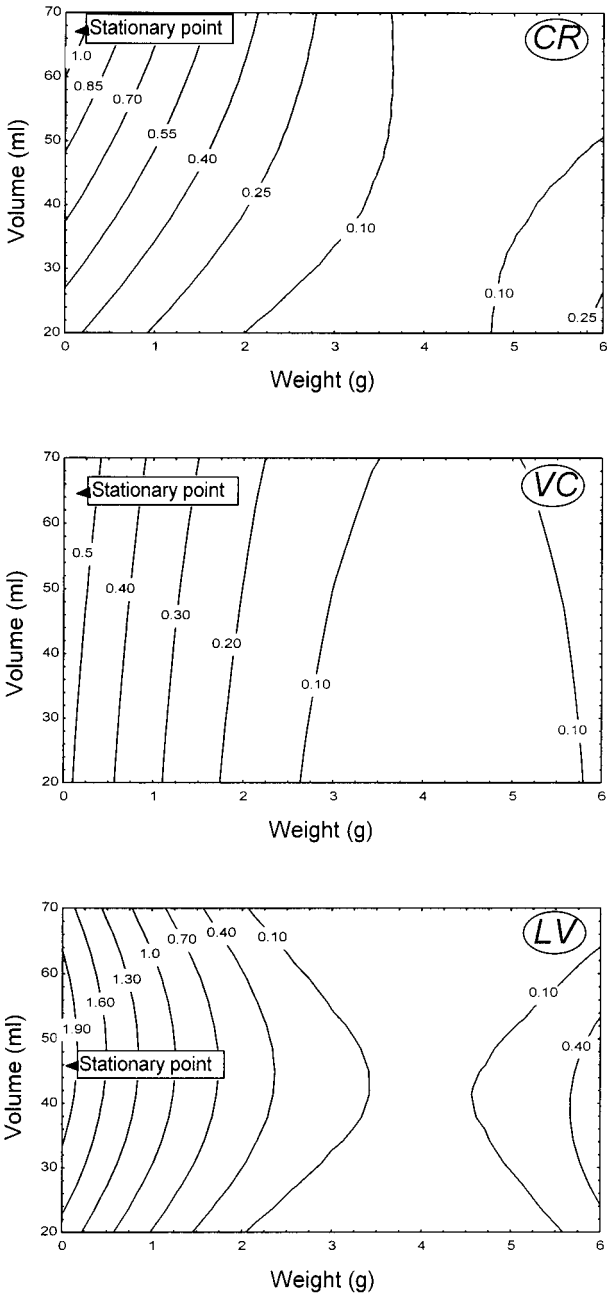

Figure 2. Contour lines of tannin content (catechin units/100 g dry material) as a function of weight sample and dilution volume using different weaning foods (CR, chicken with rice; VC, veal with carrot; LV, lamb with vegetables).

response is shown in Figure 2. In conclusion, a sample (g)/solvent system (mL) ratio for the extraction process should be considered in the range $2.6-3.8 \times 10^{-3}$ when the Terrill et al. (1992) method is used for tannin extraction from homogenized infant foods including vegetable ingredients. The literature shows a wide range of sample (g)/volume (mL) ratios used in tannin extraction, such as 2×10^{-1} (Stanley, 1992), 5×10^{-2} (Bartolomé et al., 1995), 2.5×10^{-2} (Terrill et al., 1992), 2×10^{-2} (Makkar et al., 1993), and 1×10^{-3} (Hernández et al., 1991). Thus, according to our results, it is to be expected that earlier papers using a higher sample weight/solvent ratio reported lower tannin levels.

Different authors (Naczk et al., 1992; Constantinides and Fownes, 1994) have studied the influence of different factors, including the sample/solvent ratio, on tannin extraction; in both cases, however, the experimental methodology was inadequate, because in many situations the optimum obtained by using the one-factor-at-a-time approach differs considerably from the real optimum (Joglekar and May, 1987). An inadequate experimental strategy might account for the disparity between the results obtained by both authors and those obtained here. Naczk et al. (1992) recommend a ratio (g/mL) of 5×10^{-2}. Constantinides and Fownes (1994) conclude that the sample-to-solvent ratio is the main factor causing differences in soluble polyphenol recovery and suggest a ratio (g/mL) of 2×10^{-3}, similar to the ratio proposed here, when tannins are extracted from tropical legume leaves and the solvent system used is water and 50%-aqueous methanol (v/v). In addition, in both cases, authors propose the lowest ratio included in the experimental range considered.

The negative effect of sample weight on tannin extraction (Table 4) may be due to a modification of pH in the aqueous phase during tannin extraction using the Terrill et al. (1992) method. The optimum pH for tannin−protein precipitation is found to be 0.3−3.1 pH units (Naczk et al., 1996); a larger ratio of tannin−protein precipitation implies a smaller tannin content to be hydrolyzed by acid catalysis. This hypothesis is supported by results obtained by Makkar and Becker (1996), who concluded that the recovery of tannins decreased with an increase in the pH.

In seeking to account for the negative effect magnitude exerted by sample weight on the tannin extraction process for CR, VC, and LV samples (see b_1 coefficients in Table 4), attention is drawn to the results obtained by Peng et al. (1997), who report that during the phenolysis of condensed tannins by acid catalysis (such as HCl used in the vanillin−HCl method), a set of 10 different compounds are produced as a chromogen reagent to link to the vanillin reagent. It must therefore be assumed that because each food type includes different ingredients of a vegetable origin (Table 1), the proportions of reaction products described by Peng et al. (1997) will be different for each food type. For this reason, the negative effect magnitude is different for each type, i.e., VC = 1, LV = 1.13, and CR = 1.60. In addition, it should be noted that acidified vanillin is also known to react with some flavonols, dihydrochalcones, and anthocyanins (Griffiths et al., 1998).

In conclusion, the combined use of DOE and RSM tools enables adaptation of analytical methods to foods other than those for which the method was initially described.

ABBREVIATIONS USED

DOE, design of experiments; RSM, response surface methodology; CCR, central composite rotatable; CR, chicken with rice; VC, veal with carrot; LV, lamb with vegetables; *W*, weight; *V*, volume.

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