Resveratrol and Piceid Levels in Natural and Blended Peanut Butters

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Peanut and its derivatives, especially peanut butter, are extensively consumed in many countries, mainly in the United States, which is also the major exporter of these products. *trans*-Resveratrol is present in peanuts, and recently this compound has been quantified in peanut butter. It is well-known that there are beneficial effects of *trans*-resveratrol and its glucoside, the piceid, in health. The absorption of *trans*-resveratrol has been proven in animals, and certain studies show that the absorption of some phenols is enhanced by conjugation with glucose, so that it could be possible that *trans*-piceid would be more absorbed than its aglycon (*trans*-resveratrol). In our work, we have identified the presence of *trans*-piceid in peanut butter with a new method to quantify *trans*-resveratrol and *trans*-piceid (3-β-glucose of *trans*-resveratrol). This fact is very interesting because the glucosilated form could be more easily absorbed by the intestinal gut; in this way *trans*-piceid would exercise its beneficial function more efficiently than *trans*-resveratrol. To our knowledge, this is the first time that *trans*-piceid has been quantified in peanut butter. Resveratrol and piceid contents in natural peanut butters were found to be significantly higher than those in blended peanut butters.

Keywords: *Resveratrol; piceid; peanut butter; natural; blended*

INTRODUCTION

Nowadays, there is growing evidence about the beneficial effects of *trans*-resveratrol in vitro. Animal studies have shown that *trans*-resveratrol is absorbed. However, there is a lack of studies about the physiological activity in humans. There are even fewer studies about piceid, the 3-β-glucoside of *trans*-resveratrol. Piceid has been shown to be active, inhibiting platelet aggregation (Shan et al., 1990; Chung et al., 1992; Verache-Lembege et al., 1996; Orsini et al., 1997) and inhibiting oxidation of human LDL (Merillon et al., 1996). *trans*-Piceid reduces the elevations of lipid levels (Arichi et al., 1982) and inhibits eicosanoid synthesis (Kimura et al., 1985). It seems that *trans*-piceid can be more efficiently absorbed than the aglycon, *trans*-resveratrol; according to Hollman (Hollman et al., 1995; Hollman, 1997) the absorption of some phenols from the diet is enhanced by conjugation with glucose. *trans*-Resveratrol is absorbed across the small intestine and it is conjugated to yield resveratrol glucuronide and sulfate (Andlauer et al., 2000; Kuhnle et al., 2000).

The presence of resveratrol has been extensively studied in grapes and their products: juices and wine. However, few studies have focused on peanuts and their products. Peanut and its derivatives, especially peanut butter, form part of the diet in many countries, mainly in the United States, which is the major exporter of these products. Peanut butter composition is well-known in terms of macro and micro nutrients, vitamins, and minerals. However, there is little known about the phenolic composition. The initial studies of resveratrol in peanuts focused on the presence of this compound in infested peanut (Ingham, 1976). But, more recently, the extensive study done by Sobolev and Cole (Sobolev and Cole, 1999) and the study published recently by Sanders et al. (Sanders et al., 2000) have shown the presence of *trans*-resveratrol in peanuts or their byproducts. Until now, no study has noted the presence of piceid. For this reason, we have developed a new method to quantify resveratrol and piceid in peanut butter and we have quantified these compounds in natural and blended peanut butters.

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MATERIALS AND METHODS

Standards. *trans*-Resveratrol was purchased from Sigma Chemical (St. Louis, MO). *trans*-Piceid was extracted from the roots of *Polygonum cuspidatum* as described by Waterhouse and Lamuela-Raventós (1994).

Samples. Fourteen peanut butters from the market were analyzed in duplicate. Peanut butters were kept refrigerated at 4 °C. Seven peanut butters were 100% natural, containing only roasted peanut, and the other seven were peanut butter blended with vegetable oils and/or molasses.

Phenolic Analysis by HPLC. Samples (10 g) were extracted with 100 mL of ethanol/water (80:20; v/v) at room temperature for 30 min. After centrifugation for 5 min at 3000 rpm, the supernatants were concentrated under vacuum, at temperature below 40 °C, to dryness, avoiding UV light exposure. Finally, the extracts were dissolved in HPLC mobile phase (0.4 mL of acetonitrile and 1.6 mL of acetic glacial acid 52.6% in water) to a final volume of 2 mL. Prior to the analysis by HPLC, the solution was filtered through a Whatman inorganic Anopore membrane filter (Anotop 10 plus, 0.2 μm).

A Hewlett-Packard (HP) 1050 gradient liquid chromatograph with a DAD 1050 M coupled to a Chemstation HP Rev Asterix.05.02 was used. The column, a Nucleosil 120 C_{18} (250

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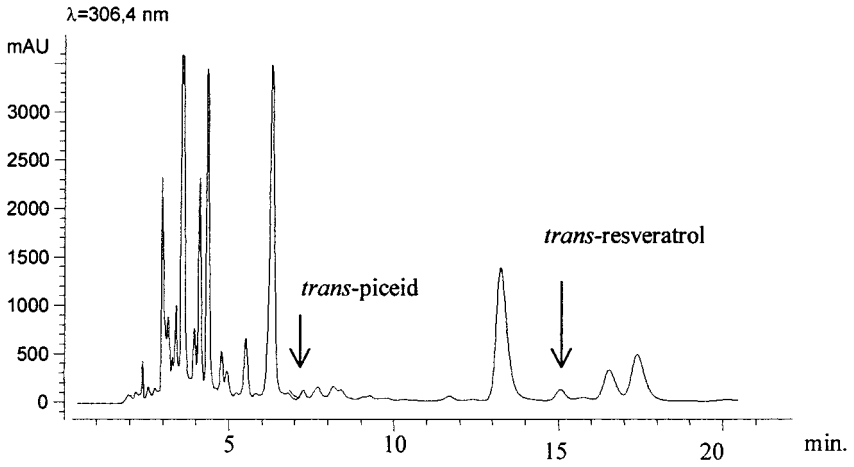

Figure 1. HPLC chromatogram of peanut butter extract at 306 nm.

Table 1. *trans*-Resveratrol and Piceid Levels in Blended (n = 7) and Natural (n = 7) Peanut Butters[a]

	trans-resveratrol (μg/g)	*trans*-piceid (μg/g)	total amount (μg/g)
blended peanut butter			
St Michael, crunchy	0.265 ± 0.003	0.089 ± 0.002	0.354 ± 0.005
Skippy, creamy	0.334 ± 0.039	0.067 ± 0.006	0.401 ± 0.045
Jif, creamy	0.335 ± 0.009	0.187 ± 0.019	0.522 ± 0.028
St Michael, smooth	0.395 ± 0.018	0.159 ± 0.005	0.554 ± 0.023
Capitán Maní, crunchy	0.339 ± 0.006	0.132 ± 0.018	0.471 ± 0.024
Capitán Maní, soft	0.671 ± 0.055	0.102 ± 0.013	0.773 ± 0.068
Sun-pat Nestlé, soft	0.522 ± 0.029	0.157 ± 0.008	0.679 ± 0.037
average, blended	0.409 ± 0.023	0.128 ± 0.010	0.537 ± 0.034
100% natural			
Monki, crunchy	0.577 ± 0.019	0.073 ± 0.010	0.65 ± 0.029
Adams, creamy	0.622 ± 0.025	0.111 ± 0.010	0.733 ± 0.035
Jori	0.671 ± 0.012	0.220 ± 0.009	0.891 ± 0.021
Glasbak Eko (unsalted)	0.753 ± 0.031	0.225 ± 0.004	0.978 ± 0.035
Horizon, crunchy	0.691 ± 0.015	0.098 ± 0.010	0.789 ± 0.025
Glasbak	0.714 ± 0.033	0.170 ± 0.002	0.884 ± 0.035
Calvé	0.534 ± 0.006	0.107 ± 0.010	0.641 ± 0.016
average, natural	0.652 ± 0.020	0.143 ± 0.008	0.814 ± 0.028

[a] Concentrations expressed in μg/g peanut butter.

× 4 mm), 5-μm particle size, was kept at 40 °C. Injection was by means of an automatic injector (HP 1050). The volume injected was 100 μL.

A constant flow rate of 1.5 mL/min was used with two solvents: solvent A was glacial acetic acid in water (52.6:900) (v/v); solvent B was 20% solvent A mixed with 80% acetonitrile. All the solvents used were of HPLC grade. We followed the conditions described previously by Romero-Pérez et al. (1999).

Identification. The chromatogram was monitored simultaneously at two wavelengths: 285 and 306 nm. The 306 nm wavelength was suitable for *trans*-resveratrol and *trans*-piceid and the 285 nm wavelength was suitable for the cis forms.

Quantification. The concentrations of *trans*-resveratrol identified were measured using the external standard method by calibration curves (standard area versus concentration in mg/L) obtained for this compound over the range of concentrations observed. The quantitation of *trans*-piceid was carried out using the *trans*-resveratrol curve because of the identical molar extinction coefficient of *trans*-resveratrol at 306 nm (Romero-Pérez et al., 1996)

Statistical Treatment. Analytical results were evaluated statistically with the STATGRAPHICS 7.0 program to evaluate by the Student's t test for the difference between natural and blended peanut butters ($p < 0.05$).

RESULTS AND DISCUSSION

HPLC Identification. Figure 1 is a chromatogram of a peanut butter extract at 306 nm. *trans*-Resveratrol and *trans*-piceid were identified by their spectra and by their retention times in comparison with standards and by the method of standard addition to the samples. However, the cis forms could not be detected in any of the samples analyzed.

Method Validation. The method was validated according to the United States Pharmacopoeia (USP 23, 1995).The parameters considered for the validation were precision, linearity, and sensitivity (limit of detection and limit of quantification).

Precision. Six aliquots of the same sample were analyzed at a time. The values are expressed by the coefficient of variation (CV %), which was 4.34% for *trans*-resveratrol and 4.10% for *trans*-piceid. These values were acceptable according to the criteria established by Horwitz (1982) for intralaboratory analysis.

Linearity. Linearity of the standard curve was studied for *trans*-resveratrol. The linearity is expressed in terms of the correlation coefficient (r). The correlation coefficient (r) was > 0.999 for *trans*-resveratrol.

Recovery. Accuracy of the method was calculated as the percentage of analyte recovered by the assay. The standard used was *trans*-resveratrol. It was added to the sample at three different levels in triplicate (9 aliquots). The percentage recoveries were 81.07 ± 2.3,

75.37 ± 0.46, and 72.31 ± 3.86 for the three levels (n = 3 for each level).

The method we propose is rapid, reliable, and simple to perform. It allows the identification and quantification of *trans*-resveratrol and *trans*-piceid in peanut butter. *trans*-Resveratrol was the major compound, with levels between 0.27 and 0.75 μg/g (Table 1), three times the levels found for piceid (0.07−0.23 μg/g). *trans*-Resveratrol content was slightly higher than the content obtained by Sbolev and Cole, 1999, (0.148−0.504 μg/g). However, they did not quantify piceid levels. Although the cis forms could be quantified in all the wines we have analyzed (Lamuela-Raventós et al., 1995; Romero-Pérez et al., 1996), they were not detected in any of these samples. Resveratrol and piceid contents in natural peanut butters were significantly higher ($p < 0.005$) than those in the blended peanut butters.

We have developed a rapid method that allows the quantitation of the trans forms of resveratrol and piceid in peanut butter that has not been previously described. Significant differences were observed in the amounts of these compounds depending on the composition of the peanut butter (natural or with added ingredients).

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