Qualitative and Semiquantitative Analysis of Dried Fruits and Seasoning Products of Paprika Using Photoacoustic Spectroscopy

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Photoacoustic spectroscopy was applied in the ultraviolet and visible regions to investigate some qualitative features of powdered paprika prepared from ripe fruits differing in color as well as of two commercial paprika spices. Absorption maxima were attributed to different pigment absorptions, especially to capsaicin and the carotenoids in all fruits and chlorophyll in the powdered fruits of green paprika. It could be shown that the highest pigment content of the commercial products is correlated to a definite particle size range of the powder. Investigations in the near-infrared region on a commercial seasoning product revealed that the intensity of an unspecific absorption in this region can be used to obtain semiquantitative information about total pigment composition.

In the last two decades, photoacoustic (PA) spectroscopy was applied to a large number of studies in distinct areas of science (Rosencwaig, 1980; Braslavsky, 1986; Vargas and Miranda, 1988; Buschmann and Prehn, 1990). Research on biological materials and also particularly on foods has advantageously employed this non-destructive technique (Belton and Tanner, 1983; Cesar et al., 1984; Haas and Jäger, 1986; Kocsányi et al., 1988; Vinha et al., 1989; Haas and Vinha, 1995).

Paprika (*Capsicum annuum*) is a fruit used, e.g., as spice to add taste to foods or as colorant for foods or cosmetics and also as raw material to the pharmaceutical industry all around the world. Pungency in paprika is caused by capsaicin, one of the most important of the mixture of various amides named capsaicinoids, which are present in paprika varieties in different amounts (Suzuki and Iwai, 1984; Somos, 1984). Therefore, generally one distinguishes by means of pungency, i.e., the content of capsaicin, between spice paprika and vegetable paprika. Spice paprika which in the majority of cases is only applied in the form of milled product and scarcely consumed raw has higher contents of capsaicin in comparison to vegetable paprika. Responsible for the color of paprika is a mixture of various carotenoids, pigments of red, orange-red, and yellow color (Somos, 1984; Khachik et al., 1992a). Studies applying TLC and HPLC on paprika have led to the identification of up to twelve different pigments of the carotenoid group with more than 40 distinct absorption peaks in the range between 400 and 520 nm (Fisher and Kocis, 1987; Biacs et al., 1989; Khachik et al., 1992a). Varying contents of red and yellow pigments are responsible for the color of the red, yellow, orange, or varicolored fruits, whereas the presence of chlorophyll determines the color of the green fruits. Chlorophyll can also be identified in some cultivars before ripeness with decreasing content by increasing maturity (Buckle and Rahman, 1979; Somos, 1984; Biacs et al., 1989). The most important carotenoids are the red-colored pigments capsanthin and capsorubin and of the remaining

orange and yellow carotenoids, e.g., β-carotene, zeaxanthin, β-cryptoxanthin, and lutein. These carotenoids can be found in all ripe cultivars (Fekete et al., 1976; Buckle and Rahman, 1979; Fisher and Kocis, 1987; Biacs et al., 1989; Baranyai et al., 1982; Ghiraldini, 1989; Khachik et al., 1992a) and in some fruits they can attain almost 70% of the total amount of extracted pigments (Fisher and Kocis, 1987). Capsanthin and capsorubin have a very strong coloring power, about 10-fold compared to that of yellow carotenoids, therefore, both pigments are largely used by the food industry to color canned meat and fish, finished food, sweets, sauce, etc. (Bertram, 1989; Ghiraldini, 1989; Marmion, 1991).

In this work PA spectroscopy was utilized in the ultraviolet (UV) and visible (vis) regions of light to investigate some qualitative spectral properties of powdered, dried fresh paprika fruits of different color. Commercial available powders of paprika products were also studied in the UV/vis region as well as in the near-infrared (NIR), in order to identify semiquantitatively the pigment content of one of the commercial products using the intensity of an absorption peak in the NIR region.

EXPERIMENTAL PROCEDURES

Apparatus. The PA spectra were recorded using an EG&G PAR model 6001 photoacoustic spectrometer equipped with a 1 kW high-pressure xenon arc lamp, which allows the recording of spectra from 200 to 2600 nm. Light modulation over the frequency range 10 Hz to 2 kHz was accomplished by electronic modulation of the lamp current. The sample cell was made of stainless steel, with a rectangular ($4 \times 8 \times 2$ mm³) sample cup. The PA signal was detected by a sensitive microphone (Brüel & Kjaer Type 4166) and "source compensated" by a real-time signal, derived from a pyroelectric detector which was exposed, *via* a fused-silica beamsplitter, to a fraction of the incident modulated light beam.

For milling of the samples, a three-dimensional oscillating mixer mill (Retsch model MM2) with grinding vessels made of agate was used, and for sieving a vibrating sieving machine (Retsch model Vibro) was employed, using ISO standard stainless-steel test sieves with wire gauges of 45, 90, 125, 180, 250, and 355 μm. In this way the samples could be separated into five fractions of different particle sizes d in following ranges: 45 μm $< d_5 \leq$ 90 μm (5th fraction), 90 μm $< d_4 \leq$ 125 μm (4th fraction), 125 μm $< d_3 \leq$ 180 μm (3rd fraction), 180

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μm $< d_2 \leq 250$ μm (2nd fraction), and 250 μm $< d_1 \leq 355$ μm (1st fraction). With an optical microscope (Olympus model BH2) the size and shape of the milled materials were examined. The samples were weighed using an electronic analytical balance (Kern Type 410–11; resolution 0.1 mg at 41 g capacity).

For the preparation of extracts of pigments an ultrasound agitator (Bandelin model Sonorex TK 52) was employed to improve the extraction efficiency.

Materials and Procedures. For three of each fruit, pericarps of red, yellow, and green ripe paprika were cut into small pieces, dried at a temperature of 40 °C during 48 h, subsequently powdered by milling, and sieved into five different grain size fractions.

Two paprika powders used in Germany as commercial seasoning products, "Sweet Paprika" and "Rosen Scharf Paprika," were investigated as received or in definite ranges of grain sizes by milling the material and subsequently passing it through standard test sieves.

A quantity of about 50 g of Sweet Paprika was sieved and separated into the five fractions with particle sizes as given above. In addition, a certain amount (about 50%) of each of the four fractions of larger particle sizes (d_1 to d_4) were reduced to the fifth fraction (size range d_5) by successive milling and sieving, to avoid or at least to reduce particle size effects on the PA signal. Pigment extraction was performed for the fifth fractions of the paprika powder using quantities in the range between 280 and 440 mg depending on the yield of each fraction reduced to this size range d_5. The remaining calibrated volume of small glass flasks with the samples, respectively, was filled with acetone to remove the soluble pigments by successively washing. For that the flasks were placed for 10 min in an ultrasound agitator and after precipitation of the remaining powder the acetone–pigment solution was carefully removed with a pipette and put into previously weighed glass ampoules to evaporate the acetone under standard conditions (temperature 20 °C) in a chimney and then weighed again to determine the remaining pigments. This entire washing procedure was performed for each sample in the glass flasks until a new filling with acetone remained optically transparent and colorless. The pigment concentration in each powder fraction was calculated of the determined net weights of the pigments relative to the weighed samples, respectively. The weight of the remaining completely washed powder was used as a control.

In principle, a modulation frequency of the incident light of 13 Hz was used for the investigations. To remove effects of photoacoustic saturation some measurements were also performed by increased modulation frequencies and by diluting the paprika powders with MgO in the ratios 1:1, 1:10, 1:50, and 1:100. All PA spectra were normalized to graphite (carbon black) as 100% absorber. The positions of absorption wavelengths were determined by applying the first and second derivatives of the absorption curves. To average several scans and to test the reproducibility, up to five measurements with new fillings of the sample cup were executed.

RESULTS AND DISCUSSION

Paprika Fruits. Spectra of dried and powdered pericarp parts of red, yellow, and green fruits of paprika (particle size range d_4) in the UV/vis region are shown in Figure 1, A–C, respectively. The absorptions of the three samples in the UV region at ca. 260 nm as well as the shoulder at about 325 nm can be attributed to the absorption of capsaicin (Suzuki and Iwai, 1984). The carotenoids predominantly reveal absorptions in the visible region contributing to the spectral response of the paprika sample in accordance with their relative concentrations in the fruit. From extinction measurements in different solvents absorption maxima of the carotenoids in the vis region are known at about the following wavelengths for, e.g., capsanthin at 460, 480, and 505 nm (Fekete et al., 1976; Parkes et al., 1986),
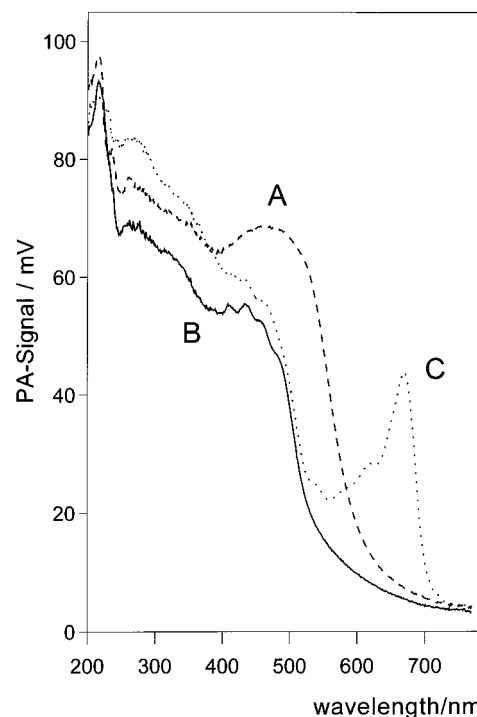

Figure 1. Photoacoustic spectrum of dried and powdered red (A), yellow (B), and green (C) paprika fruits in the UV/vis range for particles of the fourth sieving fraction (90 μm $< d_4 \leq 125$ μm).

capsorubin at 406, 480, and 510 nm (Fekete et al., 1976; Hertzberg and Liaaen-Jensen, 1985), β-carotene at 450, 466, and 494 nm (Fekete et al., 1976; DMS, 1966; Palermo et al., 1991), zeaxanthin at 430, 463, and 493 nm (Fekete et al., 1976; Hertzberg and Liaaen-Jensen, 1985; Palermo et al., 1991), cryptoxanthin at 425, 451, and 483 nm (Fekete et al., 1976; Khachik et al., 1992b), and lutein at 418, 445, and 485 nm (Goodwin and Mercer, 1972; Fekete et al., 1976). In PA studies of bulk material composed of pigment mixtures it is impossible to obtain a separation of distinct peaks by the resolution usually given as well as by signal saturation that probably is present due to strong absorption of a pigment. Therefore, in the vis region a relatively broad band results for red paprika centered at ca. 463 nm (Figure 1A), as also obtained in prior studies of red paprika using the PA technique (Kocsányi et al., 1988). The spectrum of yellow paprika (Figure 1B) reveals in the vis region four absorptions, two maxima at 411 and 435 nm and two shoulders at about 442 and 483 nm. The maximum at 411 nm can be attributed to the absorption of capsorubin, whereas the predominance of the yellow-colored carotenoids in diverse concentrations determines the maximum at 435 nm (zeaxanthin and cryptoxanthin) and the absorption at 442 nm (β-carotene, zeaxanthin, and lutein). The absorption shoulder at 483 nm must be regarded as a superposition of contributions mainly by β-carotene, capsorubin, cryptoxanthin and capsanthin in the existing pigment composition. For the green fruit special features can be seen in the vis-spectrum of the powdered material (Figure 1C). The chlorophyll absorption, not present in the red and yellow fruits, appears here with the typical absorption band of chlorophyll a at 670 nm and with the distinct shoulder of chlorophyll b on the shortwave side at ca. 630 nm (Goodwin and Mercer, 1972; Raven et al., 1988). The weak maximum at 410 nm can be attributed to capsorubin, and that at 433 nm is attributed mainly to chlorophyll a and probably to cryp-
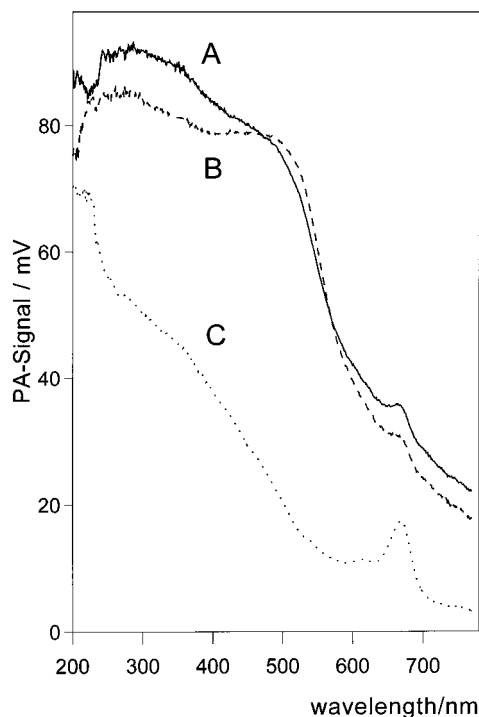
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Figure 2. Photoacoustic response of absorptions in the UV/vis range for two commercial seasoning products of paprika (A, Rosen Scharf Paprika, and B, Sweet Paprika) and (C) milled parts of the stalk of red paprika fruits.

Figure 3. Photoacoustic absorption behavior in the vis range of paprika powder diluted with MgO in the ratios 1:1 (A), 1:10 (B), and 1:50 (C).

toxanthin. Chlorophyll *b* and the yellow carotenoids β-carotene, zeaxanthin, and cryptoxanthin determine the absorption shoulder at 458 nm, whereas the red pigments capsorubin and capsanthin are responsible for the absorption at 540 nm.

Seasoning Products of Paprika. Figure 2A,B shows spectra in the UV/vis region of the commercial products Rosen Scharf Paprika and Sweet Paprika, as received, in particle size range d_4, respectively. Comparing the PA amplitudes of both spectra in the UV and vis regions it can be seen that Rosen Scharf Paprika (Figure 2A) relative to Sweet Paprika (Figure 2B) shows stronger absorptions in the UV region (the spectral range of capsaicin absorption) but reveals a reduced PA amplitude of the broad absorption of the entire set of pigments of the carotenoid group in the vis region. This can be explained by the different relative contents of capsaicin and carotenoids of the two powders because pungency of the spices increases in the order Sweet Paprika to Rosen Scharf Paprika, while their flavor declines. Therefore, the stronger absorption of Rosen Scharf Paprika in the UV region is caused by the higher content of capsaicin and the weaker absorption in the vis region due to the minor concentration of carotenoids, and *vice versa* for Sweet Paprika. By using the wavelengths of the pigment absorptions given in literature this can be derived from the photoacoustic amplitude ratios of capsaicin and carotenoid absorptions in the UV and vis, respectively, of undiluted and with MgO-diluted powders of Rosen Scharf and Sweet Paprika. The broad absorption bands for both samples in the UV/vis may be caused by signal saturation due to strong absorptions of the pigments and will be discussed later. The small maximum at about 665 nm (Figure 2A,B), which is observed only for the commercial samples but does not appear for the powdered red paprika fruit (Figure 1A), is due to small amounts of chlorophylls. The reason could be that the fruits used for the production of paprika powder either were not fully ripened or were
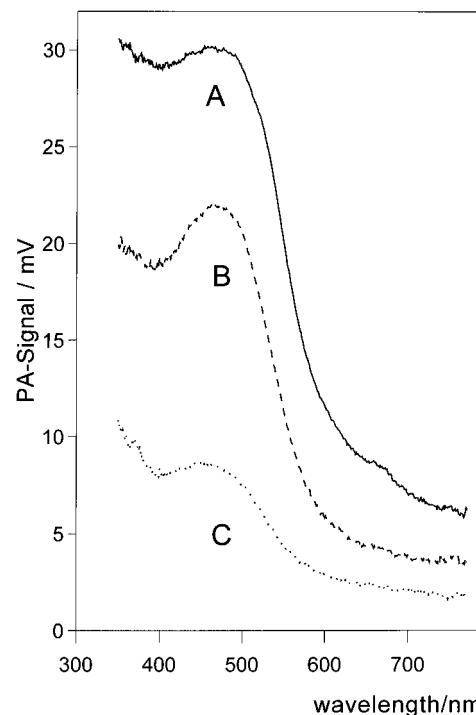
harvested and used with the stalk attached. However, the chlorophyll absorption points to a ruptured chlorophyll−protein complex because the maximum position is shifted toward shorter wavelengths compared to an intact green tissue as shown, e.g., in Figure 1C or Figure 2C. The latter spectrum was obtained by investigating only dried and milled parts of the stalk of red paprika fruits with the maximum of the typical absorption band of chlorophyll *a* close to 670 nm.

As mentioned above, the strong absorptions of the pigments causing signal saturation effects could be the reason for the broad bands of the PA spectra shown. To remove or at least to reduce saturation effects standard procedures in PA investigations are to increase modulation frequency of the incident light and to dilute the samples, e.g., with MgO. But no decrease in saturation effects could be obtained with utilizable signal-to-noise ratio of the PA signal as shown in Figure 3A−C for an investigation of three different dilutions with ratios 1:1, 1:10, and 1:50 of paprika powder with MgO. Indeed a reduction of the bandwidth is first observed, e.g., shown in Figure 3B for a ratio of 1:10, but no further improvement with increasing dilutions can be obtained, rather a reduction of the signal-to-noise ratio is observed.

It is well-known that the particle size has also a strong influence on the amplitude of PA signal. In the range between 200 and 1200 nm Figure 4 shows spectra of three fractions of Sweet Paprika separated by sieving the commercial product as received. In principle the amplitudes of the absorption bands reveal in the entire range the expected behavior, an increase with decreasing size of particles. But this is valid only in the case of a homogeneous pigment distribution for all particle size fractions. However, for paprika powder, different pigment contents in the various fractions will have an additional influence on the PA signal amplitude. Therefore, semiquantitative measurements were performed in the NIR range between 800 and 1000 nm, revealing a good signal-to-noise ratio and high reproducibility. For
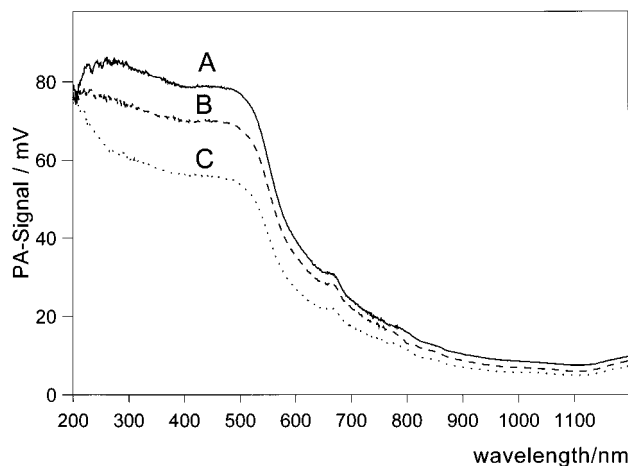
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Figure 4. Particle size dependence of the photoacoustic amplitude in the spectral range from 200 to 1200 nm for three size ranges of the commercial product Sweet Paprika: (A) 90 μm $< d_4 \leq$ 125 μm (4th fraction); (B) 125 μm $< d_3 \leq$ 180 μm (3rd fraction); (C) 180 μm $< d_2 \leq$ 250 μm (2nd fraction).
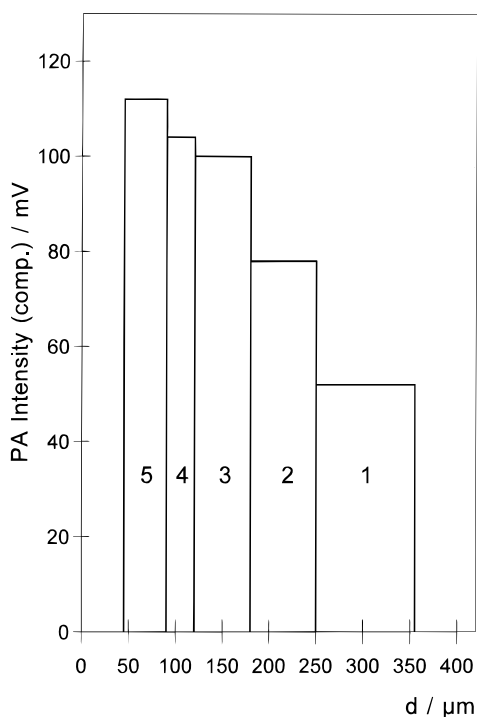

Figure 5. Histogram of the experimental determined values of PA intensity (compensated) *versus* the particle size ranges d of the five fractions obtained, e.g., by sieving the commercial seasoning product Sweet Paprika as received: 45 μm $< d_5 \leq$ 90 μm (5th fraction), 90 μm $< d_4 \leq$ 125 μm (4th fraction), 125 μm $< d_3 \leq$ 180 μm (3rd fraction), 180 μm $< d_2 \leq$ 250 μm (2nd fraction), and 250 μm $< d_1 \leq$ 355 μm (1st fraction).

quantification the magnitude of a non-specific absorption of paprika centered at 858 nm was used. Taking into account background effects, an effective amplitude [PA intensity (compensated)] of the PA signal at 858 nm was calculated by subtracting the minimum amplitude of each spectrum. The histogram (Figure 5) of the experimentally determined values of PA intensity (compensated) *versus* the particle size ranges d for Sweet Paprika as received shows an increase with reduced particle size but with a tendency to saturate for the fractions of smaller size ranges. Increasing signal saturation with decreasing particle size is an indication of a higher pigment content of the smaller particle sizes in PA investigations, i.e., in this case it must be due

Table 1. PA Intensity (Compensated) for Different Dilution Ratios of Sweet Paprika with MgO, in the Particle Size Range of the 5th Fraction (45 μm $< d_5 \leq$ 90 μm) of All Other Particle Fractions, Obtained by Subsequently Milling and Sieving Fractions 1–4, Respectively

size range origin of particles	PA intensity (compensated) in mV for dilution ratios, [sample:MgO]		
	[1:1]	[1:10]	[1:50]
1st fraction	105	41	
2nd fraction	135	46	
3rd fraction	147	48	9.2
4th fraction	135	53	10
5th fraction	125	45	10.4

to higher pigment contents in the particle size fractions 3–5. Already a visual control reveals an increase of the degree of coloring from a light to a dark red-orange for decreasing particle sizes. An optical microscopical examination of the different particle fractions shows that the larger size ranges contain more fibers and fewer pigments originating from some white-colored or almost transparent internal parts of the pericarp, which in the powdering procedure of the dried fruits tend to form larger particles. In order to exclude any effect of particle size and to attain conditions where only pigment content determines the amplitudes of the PA spectra, the first to the fourth fractions (size ranges d_1–d_4) were reduced to the fifth fraction d_5 by successive milling and sieving, respectively. Investigations of these samples in the same particle size range d_5 were performed by dilution with MgO, too, to reduce saturation of the PA signal. Table 1 shows the results of PA Intensity (compensated) for three different dilution ratios. For a 1:1 ratio saturation effects are still contributing to the PA signal of the samples originating from fourth and fifth fractions, and only for a dilution of 1:50 does saturation begin to have a negligible influence. Higher dilutions could not be investigated with utilizable signal-to-noise ratio of the PA signal. Nevertheless, the strong saturation behavior of the PA signal for particles of the same size range d_5 by originating from different size fractions, is a further indication that the highest pigment contents of paprika powder is in the smaller ranges of particle sizes d_3 to d_5. This can also be confirmed by a gravimetric determination of pigment content obtained from pigment extractions for the different fractions of particle sizes reduced to the fifth fraction, as described above. The net content (w/w) of extracted pigments varies from 16% in the fraction of larger particle size origin (size range d_3) to 23% for the smaller particle size origin (size range d_5). Altogether one can conclude that high contents of pigments are predominantly to find in ranges of particle size below about 130 μm.

CONCLUSIONS

Qualitative spectral properties of red, yellow, and green dried fruits of paprika and of two commercial available paprika spices were investigated in the UV/ vis region. In the UV region capsaicin strongly contributes to the absorption of all paprika samples, whereas the carotenoids mainly cause the absorption behavior in the vis range. A broad absorption band is obtained in the vis for the powder of the red fruit, whereas for the yellow and green fruits some distinct absorption maxima of carotenoids can be observed and in addition for the green fruit the typical absorptions of chlorophyll appear. The different ratios of the PA amplitudes in the UV and visible regions of the com-

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mercial seasoning products give information about the various contents of the carotenoid and capsaicin pigments and hence about pungency.

The amplitudes of the PA signal show the expected effects of saturation and dependence of particle size but in particular saturation only in a definite particle size range. Investigations of a commercial paprika powder milled and sieved subsequently to the smallest particles size range and diluted with MgO showed that the highest pigment content is correlated to particle sizes of the powder below about 130 μm. Experiments in the NIR region on a commercial seasoning product revealed that the intensity of an unspecific absorption in this region can be used to obtain semiquantitative information about total pigment composition.

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