Effect of Processing of Paprika on the Main Carotenes and Esterified Xanthophylls Present in the Fresh Fruit

M. I. Mínguez-Mosquera,* M. Jarén-Galán, and J. Garrido-Fernández

U.E. Biotecnología de Alimentos, Instituto de la Grasa y sus Derivados (CSIC), Avenida Padre Garcia Tejero 4, Sevilla 41012, Spain

Pigments have been monitored during the processing of paprika. Considerable variety-dependent differences were found in pigment stability and, consequently, in the quality of the respective paprikas. In the global process, the loss in the concentration of pigments caused by the addition of seed during the milling was greater than that caused by degradation. The steps of drying and milling did not affect all of the pigments equally. Individually, the yellow pigments were the most unstable, particularly β-carotene. The red pigments were highly stable, with minimal degradation due to the process.

INTRODUCTION

Forthcoming regulations will tend to restrict the use of foodstuff colorants exclusively to natural compounds, while artificial compounds will disappear progressively from the lists of acceptable colorants (Chambolle, 1992). It will therefore be necessary to study the characteristics of these natural compounds, so that the industry can develop profitable processes that guarantee homogeneous stable products. The results will affect the interests of both the industry and the consumer.

One of the most traditional natural foodstuff colorants, widely used at the industrial level, is paprika, whose quality lies exclusively in its coloring capacity. Increasing demand for this product has forced the industrialization of its processing, which until recently has been totally a craft industry. To evaluate the classic parameters of paprika quality (and others not considered before, such as provitamin A value or stability), a detailed study is necessary of the content and type of pigments present in the fruit and in the paprika.

Zapata et al. (1992) explain the different processes used to obtain paprika according to regions and varieties. Fruits are picked after full ripeness, to reduce drying costs. The fruit is left to overripen on the plant or is stored temporarily to produce a natural loss of moisture, resulting in a softening of the flesh which helps subsequent drying. During this stage, cracks form in the surface of the fruit, and even localized infections occur, creating areas which show up during drying as colorless marks. This process is at present carried out under countercurrent, achieving a quick cheap drying of the fruit. Lastly, the dry pepper is triturated to the required granulometry. During this milling, the fruit is subjected to heating from friction and bruising from the mill hammers. To improve the appearance and homogenization of the sample, it is normal practice during this stage to add a certain percentage of seed from the fruit (Sancho Gómez, 1962). This gives a homogeneous quality throughout the production period, and the presence of oil from the seed aids trituration and enhances the gloss of the final product, paprika, but its coloring capacity is considerably reduced. It is stored until cool and then packaged.

The sequence of industrial operations to obtain paprika may destroy part of the components initially present in the fruit, in particular the fraction including the carotenoid pigments (Lease and Lease, 1956, 1962), which are those exclusively responsible for the commercial quality of paprika (Salmerón Salmerón, 1973). Degradative metabolic processes associated with overripening (Gross, 1991), microbial metabolism (Mínguez Mosquera et al., 1989), oxidative processes beginning during or set off by heating, and later potentiated by the presence of highly unsaturated oils (Kanner et al., 1976, 1977) and the increase of surface due to milling, may cause significant loss of pigments and stability of the final product, even under the mildest conditions.

A previous work (Mínguez Mosquera et al., 1992) showed that in peppers from the paprika-producing region of Murcia (Spain) ratios between the main pigment fractions remained constant within the normal limits for natural products, notwithstanding variety. This was also true for paprikas obtained in the laboratory. However, when commercial paprikas were monitored, samples were detected with abnormal ratios compared with those found in the laboratory. It was shown that this was due to degradative changes, mostly in the pigments of the yellow fraction.

The present work studies the possible effects of industrial dehydration and milling on the initial pigment content in ripe red peppers. The study is based on individual quantitative changes of fruit pigments during those stages. Especially interesting are the ratios between components, which permitted a valid comparison and monitoring of the pigment evolution, avoiding variables such as moisture or different natural pigment content. This method evaluates both the stability of each pigment during processing and the quality of the raw material by variety, allowing preselection to obtain a good paprika.

MATERIALS AND METHODS

Raw Material. Overripe fruit of two varieties of pepper (*Capsicum annuum* L.), *Bola* and *Agridulce*, were used, before and after dehydration during 8 h at 60 °C, in industrial drying tunnels under countercurrent. The paprikas obtained from each variety were analyzed at the end of the process and after 9 months conserved in darkness at 10 °C in a cold chamber. All samples were of industrial origin, supplied by Netasa of Plasencia and Tiburcio Pérez of Cuacos de Yuste, from the paprika-producing region of La Vera, Cáceres (Spain).

For the analysis, samples were weighed in quadruplicate: 10 g of sample for fresh fruit and 1.5 g for dried fruit and paprika. The weighed samples were frozen at −30 °C until extraction of pigments. For the extraction, and to homogenize the raw material as much as possible, some 10 mL of water was added to the dehydrated samples, dry pepper, and paprika, to make their content similar to that of the fresh fruit. The extraction was performed after rehydration.

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Table I. Calculation of the Coefficient of Extinction (CE) at 1% in Esterified Pigments

pigment	wavelength, nm	solvent	saponified MW	saponified CE[a]	esterified MW	esterified CE
β-carotene	450	acetone	536.88	2503		
cryptoxanthin	452	petroleum ether	552.88	2369	790.88	1656
zeaxanthin	452	acetone	568.88	2340	1056.54	1264
capsanthin	518	acetone	584.88	2069	1007.24	1169
capsorubin	489	acetone	600.88	2200	1023.24	1258

[a] Davies and Köst (1988).

Extraction, Separation, and Identification of Pigments. All of these processes have been described in detail in previous works (Mínguez Mosquera et al., 1992; Mínguez Mosquera and Hornero Méndez, 1993). The pigments were extracted with acetone until the filtrates were colorless. The acetone extracts were combined and transferred to ethyl ether. The solvent was evaporated to dryness and the dry residue collected in 10 mL of acetone.

Pigments were separated by TLC with silica gel 60G support and hexane/ethyl acetate/ethanol/acetone (95:3:2:2) as developer (Mínguez Mosquera et al., 1984). Before and after saponification, each purified pigment was identified by the usual techniques: localization of absorption maxima in the spectra in different solvents and peak ratios, functional group tests (conversion of the 5,6-epoxide group into 5,8-furanoid in acid medium, acetylation of free hydroxyl groups, and reduction with borohydride of ketone groups), and infrared spectrum (Moss and Weedon, 1976). The identification tests were repeated in each extract.

Quantification of Pigments. In each pigment, the quantification was by individual scraping and elution with the appropriate solvent to a final volume allowing the correct spectrophotometric measurement. Following the studies of Hornero-Méndez et al. (1992) on the determination by GC of the degree of esterification and percentage of fatty acids involved in the esterification of each pigment, the appropriate E_0 of each pigment was calculated for the correct quantification at 1%.

Each pigment extract was chromatographed in duplicate, obtaining eight results per pigment, variety, and stage of processing.

Apparatus Used: Hewlett-Packard UV–vis spectrophotometer photodiode array, Model 8450, provided with a Hewlett-Packard recorder, Model 7225 A; Perkin-Elmer 782 IR spectrophotometer, with computer, Model 3600.

RESULTS AND DISCUSSION

Pigments Monitored in Fresh Fruit, Dried Fruit, and Paprika. Chromatographic development by TLC of the pigment extracts of fruit of different varieties of pepper and from different stages of processing showed similar characteristics.

The pigments identified for this study are in accord with those found previously (Mínguez Mosquera et al., 1992) and comprise the hydrocarbon fraction: band 1, made up of phytoene, phytofluene, β-carotene, and ζ-carotene; and the fraction that includes the principal esterified xanthophylls—both red and yellow—and which are detailed as band 2, cryptoxanthin, band 3, zeaxanthin, band 4, capsanthin, and band 5, capsorubin. These pigments, by their nature or degree of esterification, are those of lowest polarity and together make up more than 60% of the pigment content of pepper.

The qualitative similarity and the identification test shows that there is no formation of new carotenoids during processing of the pepper, by either biosynthetic or degradative pathways, nor is there variety exclusiveness in these pigment fractions.

Pigment Content during the Steps of the Processing of Paprika. The hydrocarbon carotenoid pigments were quantified globally, using the β-carotene coefficient—the major pigment. For each xanthophyll, the corresponding E_0 was used, calculated according to the esterification conditions (Table I). For this calculation, it was previously confirmed that esterification did not affect the molar coefficient of extinction but did influence that calculated at 1%, by the increase in weight of the pigment. Quantification was carried out with reference to dry material. The mean values and limits of confidence are shown in Table II.

Capsanthin was in all cases the major pigment, followed by β-carotene in fresh fruit and by capsorubin once the processing of the fruit to obtain paprika had begun. Between varieties, *Agridulce* always showed a higher pigment content. In fresh fruit, the differences in concentration of the yellow pigments in both varieties were estimated around 8% for each one, while they were around 40% for the red pigments. After drying, the difference in yellow pigments became unequal, with the *Bola* variety having only some 35% of the pigment content of the *Agridulce* variety. The differences found in red pigments remained similar to those of fresh fruit.

During milling all of the pigments suffered a sharp decrease, which could be attributable to two factors: either milling is a drastic treatment that largely degrades the pigments or there was a dilution by the addition of inert material. Possibly in reality both factors play a part. During storage, with respect to varieties, the product from *Agridulce* had a higher yellow pigment content, with up to triplicate with respect to *Bola*, as in the case of

Table II. Concentration of the Main Carotenes and Esterified Xanthophylls in Fruits of Two Varieties of Pepper during the Steps of the Processing of Paprika

sample	pigment concn, mg/kg of dry matter: β-carotene	cryptoxanthin	zeaxanthin	capsanthin	capsorubin
			Var. *Bola*		
pepper					
fresh	685.4 ± 24.2	217.4 ± 9.8	295.6 ± 8.2	1891.2 ± 38.4	364.3 ± 123.9
dry	280.5 ± 10.7	92.0 ± 3.9	90.9 ± 3.8	1934.4 ± 59.3	499.0 ± 19.7
paprika					
recent	125.6 ± 2.8	64.4 ± 2.9	83.9 ± 3.5	1160.6 ± 23.3	230.6 ± 12.2
stored	57.8 ± 6.4	27.7 ± 3.7	54.0 ± 1.8	1070.9 ± 19.9	177.3 ± 11.0
			Var. *Agridulce*		
pepper					
fresh	850.3 ± 40.8	297.9 ± 12.6	350.0 ± 9.3	2857.7 ± 44.1	602.0 ± 32.4
dry	719.4 ± 22.1	280.9 ± 17.5	295.9 ± 19.9	2344.3 ± 59.6	582.8 ± 38.6
paprika					
recent	263.0 ± 12.0	110.5 ± 4.3	150.1 ± 4.0	1344.7 ± 23.7	275.4 ± 8.5
stored	184.2 ± 11.7	74.2 ± 4.2	100.1 ± 8.4	1185.0 ± 16.8	246.3 ± 7.1

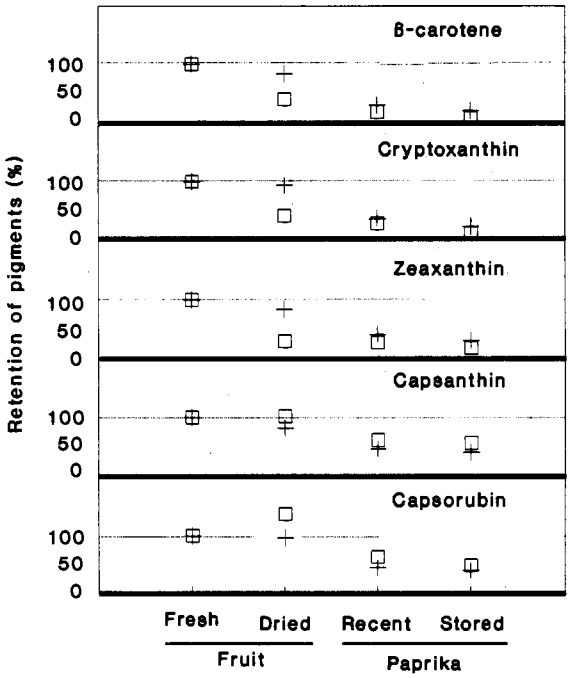

Figure 1. Influence of the paprika processing on the retention of the initial pepper pigment content in fruits from *Bola* (□) and *Agridulce* (+) varieties.

β-carotene. The changes in capsanthin and capsorubin were approximately equal.

Figure 1 shows the retention percentage of color in each step of the process. The retention was different for each pigment, although as a general rule there was a greater loss of the yellow fraction, above all of β-carotene. This effect was much stronger in the case of the *Bola* variety.

For the *Bola* variety, an unexpected result was the increased concentration of capsanthin and capsorubin in the step from fresh fruit to dry. In principle, this is not physiologically plausible, as it is assumed that during the drying stage the biosynthetic pathways are interrupted and the catabolic and degradative reactions are enhanced. In capsanthin, the small increase fell within what could be described as experimental error, but with capsorubin this argument is not possible as the increase was approximately 40%. This increase can have only two causes: (a) Biosynthesis of carotenoids takes place during the drying process. (b) A certain amount of pigment initially present in the fruit, with a low degree of esterification and thus not among the pigments quantified, is esterified during the processing and thus quantified in the second stage of the process. In the face of lack of further proof confirming either hypothesis, neither of them can be discarded.

In the total balance of pigment material from the step fresh fruit to paprika, the retention of carotenoids in the *Bola* variety was close to 40%. Capsanthin is retained some 60%, and capsorubin also showed a retention close to 50% of its initial concentration, but it must be borne in mind that this pigment is present in smaller amount in the fresh fruit than in the dry, so that the loss with respect to the fresh fruit does not reflect the real alteration. In the *Agridulce* variety, the mean retention of pigmentation was some 36%.

From a comparison of each step of processing with its immediate predecessor, it is observed that in the *Bola* variety the same percentage of β-carotene was retained in all stages, in a proportion of some 50%. Thus, there is no particularly critical step for the alteration of this pigment. From the initial content of cryptoxanthin, there was a loss of around 50% during the storage, while during milling the concentration was reduced only some 30%. Zeaxanthin was degraded in the drying stage, remaining practically unaltered during milling. The increase in concentration of capsanthin and capsorubin during drying was lost later in milling and the subsequent storage, but to a lesser extent than in the above-mentioned pigments. There was minimal degradation of capsanthin in both stages, making it the most resistant.

Table III. Pigment Composition of Pepper Seed

pigment	concn, mg/kg of dry matter	pigment	concn, mg/kg of dry matter
β-carotene	1.45	capsanthin	0.85
cryptoxanthin	0.80	capsorubin	0.00
zeaxanthin	1.35		

In the *Agridulce* variety, drying affected all of the pigments, with an average retention of 85%. Milling caused a sharp fall in pigment retention, ranging between 40 and 60%, with maximum loss in the yellow carotenoids. This tendency was repeated during storage, with maximum values of 40% and minima of 20%. Except in the drying stage, the most resistant pigment was always capsanthin.

The fact that there was a minimum loss in concentration close to 40% of all the pigments during milling can thus be attributed directly to the addition of seed—a dilution with inert matter. Given that capsanthin in the other steps has been the most stable of all the pigments under study, its decrease during milling can be considered insignificant compared with that induced by dilution; thus, the contribution of inert matter was some 44%. Consequently, it can be interpreted that in the 64% loss of β-carotene, 44% was due to dilution and only 20% to destruction during processing. In β-carotene of *Bola*, and using the same reasoning, the dilution from addition of seed was 40% and the additional loss from processing 15%. In *Agridulce*, the effect of milling on the pigments was more drastic, apart from the effect from higher dilution with seeds.

From the foregoing, in paprika from the *Bola* variety (which has a lower concentration of pigments in its fruits), the addition of seed in the last phase of processing should be in the proportion estimated, so that the resulting product has apparently similar characteristics to those of the product from the *Agridulce* variety.

In the case of zeaxanthin (*Bola* variety), there should be a loss from dilution as in the other pigments. This did not happen. Initially, this was attributed to the possible presence of zeaxanthin in the seed, which would cause the opposite effect. A detailed study of the pigment composition of pepper seed is summed up in Table III. As initially supposed, zeaxanthin and β-carotene were the major pigments, but their contribution can in no way be considered sufficient to explain the increase in amount of zeaxanthin.

Comparative Stability of Varieties. Changes in Pigment Ratios. From the pigment cocentration, the ratios between both individual pigments and fraction groups were calculated and are shown in Table IV. The fractions were obtained by adding the individual concentration of pigments showing yellow and red coloring. Changes in the ratios during processing demonstrate which are, comparatively, the most labile carotenoids and which stage of the processing is the most critical, as the samples analyzed are all from industrial sources.

The ratio of β-carotene to any of the other pigments decreases as processing of paprika advances, indicating clearly that it is the most labile pigment present in the

Table IV. Comparative Stability of Pigments in Different Pepper Varieties: Changes in Pigment Ratios

	var. *Bola*				var. *Agridulce*			
	fruit		paprika		fruit		paprika	
ratio	fresh	dry	recent	stored	fresh	dry	recent	stored
β-carotene/								
cryptoxanthin	3.15	3.05	1.95	1.97	2.85	2.56	2.38	2.47
zeaxanthin	2.32	3.08	1.50	1.08	2.43	2.43	1.75	1.84
capsanthin	0.36	0.15	0.11	0.05	0.30	0.31	0.20	0.16
capsorubin	1.88	0.51	0.61	0.33	1.41	1.23	0.96	0.75
cryptoxanthin/								
zeaxanthin	0.74	1.01	0.77	0.55	0.85	0.95	0.74	0.74
capsanthin	0.11	0.05	0.06	0.03	0.10	0.12	0.08	0.06
capsorubin	0.60	0.17	0.28	0.17	0.49	0.48	0.40	0.30
zeaxanthin/								
capsanthin	0.16	0.05	0.07	0.05	0.12	0.13	0.11	0.08
capsorubin	0.81	0.17	0.36	0.30	0.58	0.51	0.55	0.41
capsanthin/								
capsorubin	5.18	3.53	5.03	6.05	4.75	4.02	4.88	4.81
total yellow pigments/								
β-carotene	1.75	1.64	2.17	2.44	1.75	1.81	2.00	1.96
cryptoxanthin	5.55	5.00	4.16	4.76	5.00	4.54	4.76	4.76
zeaxanthin	4.00	5.00	3.22	2.63	4.34	4.34	3.44	3.57
capsanthin	0.63	0.24	0.23	0.13	0.52	0.55	0.39	0.30
capsorubin	3.33	0.84	1.19	0.80	2.50	2.22	1.88	1.45
total red pigments/								
β-carotene	3.37	9.09	11.11	20.00	4.00	4.00	6.25	7.69
cryptoxanthin	10.00	25.00	20.00	50.00	11.11	10.00	14.28	20.00
zeaxanthin	7.69	25.00	16.60	25.00	10.00	10.00	11.11	14.28
capsanthin	1.19	1.28	1.20	1.16	1.20	1.25	1.20	1.20
capsorubin	6.25	4.54	5.88	7.14	5.88	5.00	5.88	5.88
total red/total yellow	1.88	5.36	5.08	8.82	2.31	2.26	3.09	3.99

fresh fruit. This lability is particularly marked in the *Bola* variety, in which the ratio β-carotene/capsanthin decreased 86% following storage, while in *Agridulce* it decreased only 46%. This result mirrors that from calculation of the percentage loss.

The ratio between cryptoxanthin and zeaxanthin remained approximately constant, and the ratios with capsanthin decreased gradually with progression of processing. Thus, it can be concluded that both are equally unstable, although much less than β-carotene. In the *Bola* variety, the decrease in these ratios was again greater than that in the *Agridulce* variety, implying also higher lability in these pigments in the *Bola* variety.

In the red fraction, the ratio between capsanthin and capsorubin was much more constant than those found between the yellow compounds, although there was a slight tendency to rise (16% in *Bola* and 1% in *Agridulce*). This demonstrates a higher stability in capsanthin. This constancy in behavior was independent of variety for the first time.

On the other hand, the ratio between the total fractions of red and yellow pigments was calculated. This ratio increased with each step of processing. The yellow pigments again showed greater lability, particularly those of the *Bola* variety. In fresh fruits, this ratio was 1.8 for *Bola* and 2.31 for *Agridulce*, indicating the higher initial proportion of yellow pigments in the fresh fruit of *Bola*. In the paprika this ratio was 5.08 for *Bola* and 3.09 for *Agridulce*. The latter value is in agreement with that of the paprikas of Murcian varieties obtained in the laboratory (Mínguez Mosquera et al., 1992).

A slight tendency to rise was observed in the *Agridulce* variety, indicating a loss of yellow pigments. However, in *Bola* this loss was massive, but the reason for the greater lability of the carotenoids in this pepper is unknown. Obviously, the cause must be absolutely intrinsic to the variety, as in both cases the fruits were subjected to the same processing.

It is clear that there are marked differences in pigment stability depending on variety, in addition to the already known intrinsic differences in the quantitative composition of pigments. The broadening of this study to take in the remaining varieties used for paprika could allow *a priori* selection of the raw material and type of processing to obtain a product with predefined characteristics in coloring capacity and color stability.

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