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Phenolic Compounds Analysis in the Determination of Fruit Jam Genuineness

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The flavonoids and other phenolics present in different commercial jams of apricot, peach, plum, strawberry, sour orange, apple, and pear have been studied, and the characteristic compounds for each different jam identified. As a general rule these substances do not differ from those found in the natural fruits. Every jam type generally has a distinctive flavonoid pattern characterized by the occurrence of one or more markers. The high sugar and pectin contents of fruit jams constitute an important problem in the extraction of phenolic compounds for analysis. This problem has been solved by filtration of the diluted original extracts through Amberlite XAD-2 resin columns. The characteristic HPLC phenolic profiles for each jam type are presented. This technique can be useful in the determination of mixtures of jams in genuineness studies. This would be especially interesting in the detection of apple in apricot, peach, or pear jams, as well as in the detection of apricot in peach jam.

INTRODUCTION

Flavonoid analysis has recently been used in studies of the origin of wines (Etievant et al., 1988; Tomás-Lorente et al., 1989), fruit juices (Rouseff et al., 1987; Galensa, 1988; Rouseff, 1988), bee pollen (Tomás-Barberán et al., 1989), and honey (Amiot et al., 1989; Ferreres et al., 1991). This is mainly due to the fact that flavonoids are very suitable compounds for chemotaxonomic studies since every plant species (or even cultivar) has a characteristic flavonoid pattern which allows its differentiation (Harborne and Turner, 1984).

As part of our research program on the study of flavonoids and other phenolics from jams for typification purposes and to detect fraudulent mixtures, we have studied these substances in different commercial fruit jams.

In the investigation of phenolic compounds from jams, a major problem arises in the preparation of samples for HPLC analysis, since the large amounts of sugars and pectins make difficult the extraction of these substances. This is probably the reason why no study on flavonoid compounds from jams has been published to date, to the best of our knowledge.

The aim of the present work is to describe an easy technique which enables the analysis of flavonoids and other phenolics from fruit jams and to apply this technique in genuineness determinations.

MATERIALS AND METHODS

Material. Different commercial fruit jams available in the Spanish market were analyzed: six from apricot and peach and three from plum, strawberry, sour orange, apple, and pear. The origins of the different samples (where processed) are shown in Table I. Commercial apricot jams were generally obtained from Búlida cv. fruits, peach jams from Sudenell amarillo de agosto or Maruja cv. fruits, and plum jams from Reina Claudia cv. fruits. Strawberry jams were from Douglas or Charter cv. fruits, sour orange jams were obtained from fruits of *Citrus aurantium* (Seville orange), apple jams were from Golden Delicious cv. fruits, and pear jams were obtained from Williams or Limonera cv. pears.

Phenolics Extraction. The phenolic compounds present in the available jams (200 g) were exhaustively extracted by stirring with 400 mL of 80% methanol for 18 h at room temperature and filtered. The filtrate was concentrated under reduced pressure at 35 °C until all of the methanol had been removed. The flavonoids present in the remaining aqueous extract (ca. 50 mL) were then completely extracted (three times) with 1-butanol (50 mL) by agitation in a separation funnel.

Sample Preparation. Butanol extracts were taken to dryness under reduced pressure (50 °C) and the residues

Table I. Commercial Jams Analyzed

sample[a]	origin (where processed)	date of manufacture
apricot		
1	Murcia (Spain)	1989
2	Murcia (Spain)	1990
3	Valladolid (Spain)	1990
4	Valladolid (Spain)	1987
5	Sevilla (Spain)	1987
6	Lana d'Adige (Italy)	1988
peach		
1	Murcia (Spain)	1990
2	Murcia (Spain)	1990
3	Valladolid (Spain)	1989
4	Valladolid (Spain)	1990
5	Sevilla (Spain)	1987
6	Lana d'Adige (Italy)	1988
plum		
1	Murcia (Spain)	1990
2	Valladolid (Spain)	1990
3	Bretonoux Biars (France)	1988
strawberry		
1	Murcia (Spain)	1990
2	Valladolid (Spain)	1990
3	Bretenoux Biars (France)	1988
sour orange		
1	Murcia (Spain)	1990
2	Sevilla (Spain)	1988
3	Bretenoux Biars (France)	1988
apple		
1	Murcia (Spain)	1990
2	Warburg (Germany)	1989
3	Lubersac (France)	1987
pear		
1	Murcia (Spain)	1989
2	Murcia (Spain)	1990
3	Murcia (Spain)	1989

[a] Every sample of the same fruit jam was processed by a different company.

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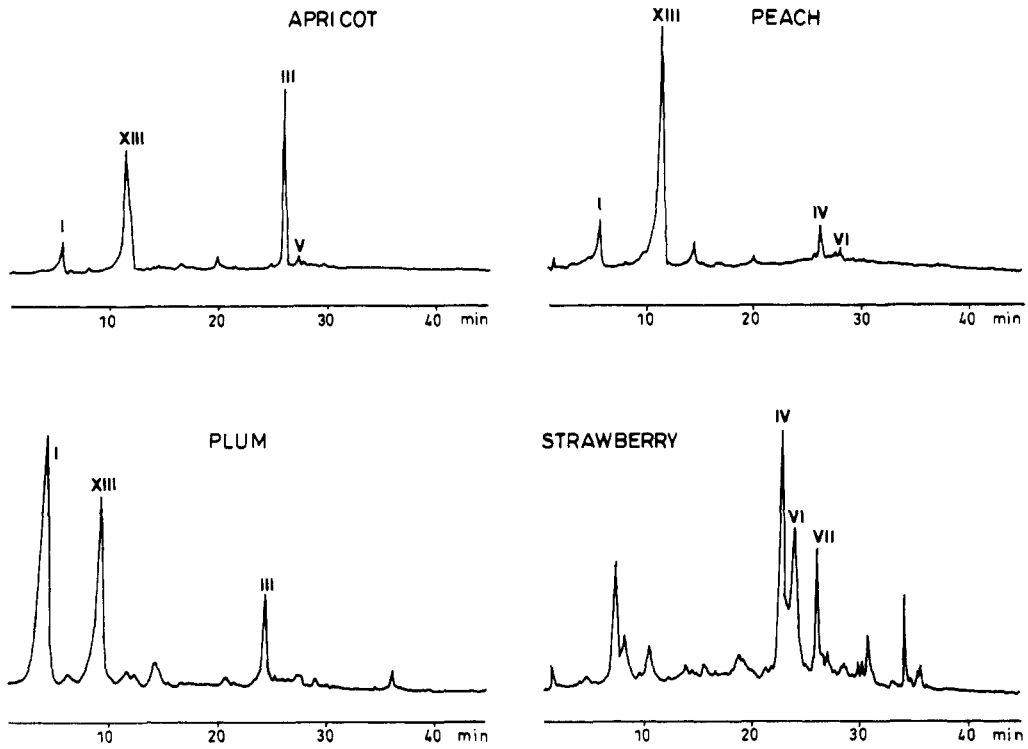

Figure 1. HPLC chromatographic profiles of characteristic phenolics from apricot, peach, plum, and strawberry jams. Detection was achieved at 350 nm. (I) Caffeic acid; (III) rutin; (IV) quercetin 3-glucoside; (V) kaempferol 3-rutinoside; (VI) kaempferol 3-glucoside; (VII) kaempferol 7-glucoside; (XIII) chlorogenic acid. In strawberry, IV = quercetin 3-glucoside + quercetin 3-glucuronide and VI = kaempferol 3-glucoside + kaempferol 3-glucuronide.

redissolved in acid water (pH 2 with HCl) (50 mL). These aqueous extracts were then filtered through an Amberlite XAD-2 resin column (40 × 2 cm) (Fluka 20–50 mesh) with a solvent flow of 10 mL/min. Flavonoid compounds remained in the column while sugars, pectins, and other polar compounds eluted with the aqueous solvent (no flavonoid was detected to elute with this solvent). The column was then washed with acid water (ca. 100 mL) and subsequently with neutral distilled water (ca. 300 mL). The flavonoid fraction was eluted with methanol (ca. 300 mL) and concentrated under vacuum and redissolved in 2 mL of methanol (except the sour orange samples which were redissolved in 4 mL).

HPLC Analysis. Ten microliters of the flavonoid fraction was analyzed by HPLC (Merck Hitachi L-6200 intelligent pump equipped with photodiode array detector Merck Hitachi L-3000) with a Lichrochart 100 RP-18 reversed-phase column (12.5 × 0.4 cm) (particle size 5 μm) using as mobile phase water–formic acid (95:5) (solvent A) and methanol (solvent B). Elution was performed at a flow rate of 1 mL/min using a gradient starting with 5% B and increasing B to levels of 30% B at 20 min, 50% B at 25 min, and 80% B at 35 min. Detection was achieved at 280 and 350 nm. The UV spectra of the different phenolics were recorded with a photodiode array detector.

Phenolic Compounds Identification. The different compounds had been characterized by standard UV, chromatographic, electrophoretic, enzymatic, and hydrolytic techniques (Mabry et al., 1970; Harborne, 1973, 1989; Markham, 1981) and wherever possible by chromatographic comparisons with authentic samples as in the case of chlorogenic acid (Sigma), caffeic acid (Sigma), phloridzin (Sigma), rutin (Roth), neohesperidin (Roth), naringin (Roth), 1,4-dihydroxybenzene (Sigma), quercetin 3-glucoside from *Helianthemum lavandulaefolium* (Ferreres and Tomás-Lorente, 1982), kaempferol 3-rutinoside from *Capparis spinosa* (Tomás and Ferreres, 1976), and kaempferol 3-glucoside from *Vicia faba* (Tomás-Barberán et al., 1991).

RESULTS AND DISCUSSION

HPLC Sample Preparation. The main problem in HPLC analysis of flavonoid from jams is the large amounts of sugar and pectins they contain, which make rather difficult flavonoid extraction and preparation of samples for analysis. The use of the resin Amberlite XAD-2 for the removal of sugars and polar compounds from plant extracts, to obtain a purified phenolic compound fraction, has been reported (Rosler and Goodwin, 1984). This technique has recently been used with success in the analysis of flavonoids from honey (Ferreres et al., 1991). In the present work we have used this filtration technique for the analysis of phenolic compounds from fruit jams.

The sample preparation technique described under Materials and Methods allows the removal of sugars, pectins, and polar compounds from jam and juice extracts and afforded a purified flavonoid fraction in only one step, by means of filtration through an Amberlite XAD-2 column. These extracts were then HPLC analyzed; the results are given below.

The recovery of flavonoid aglycons and glycosides after this filtration has previously been tested (Tomás-Barberán et al., 1992). A number of solutions of 10 mg of rutin and 10 mg of quercetin in 100 mL of water with 20% sucrose were filtered through the Amberlite XAD-2 column. All sugars had been removed by washing with water (no flavonoid was detected in significant amount in these eluates), and in all cases more than 90% of rutin and quercetin was recovered by eluting with methanol (Tomás-Barberán et al., 1992).

The reproducibility of the extraction technique was qualitatively assayed by extracting and HPLC analyzing nine replicate samples of apricot jam 1 (Table I), and a single phenolic profile was obtained in all cases.

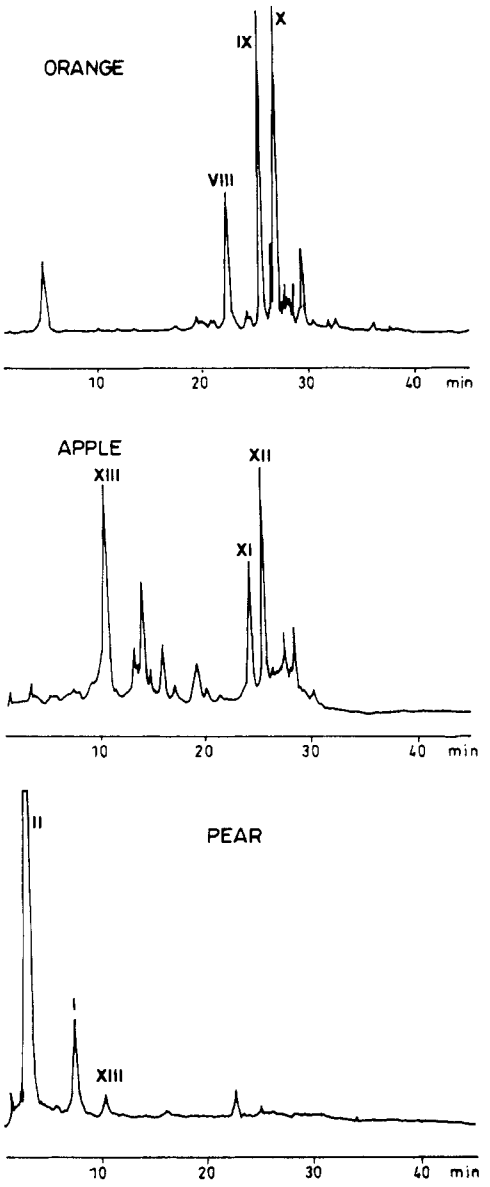

Figure 2. HPLC chromatographic profiles of phenolics from sour orange, apple, and pear jams. Detection was achieved at 280 nm. (II) Arbutin; (VIII) neoeriocitrin; (IX) naringin; (X) neohesperidin; (XI) phloretin 2′-xyloxylglucoside; (XII) phloretin 2′-glucoside; (XIII) chlorogenic acid.

HPLC Phenolic Profiles of Fruit Jams. The phenolic extracts obtained from the available commercial jams by filtration through the Amberlite XAD-2 column were HPLC analyzed under the same analytical conditions using a photodiode array detector to record the UV spectra of the different compounds and to assess the optimal wavelengths for the detection of characteristic phenolic profiles for each type of fruit jam. Thus, apricot, peach, plum, and strawberry jam extracts show the most characteristic phenolic profiles at 350 nm, since their characteristic phenolics are flavonols which are easily detected at this wavelength (Figure 1). However, apple, pear, and sour orange jam extracts should be detected at 280 nm, where flavanones, dihydrochalcones, and arbutin, which are the characteristic compounds of these fruits, have the maximum absorption (Figure 2). These results show that the HPLC phenolic profiles could be used as "fingerprints" to detect qualitative differences in a jam composition. When different commercial jams of the same fruit are analyzed by this technique, the same flavonoid profiles are obtained for each sample (Tables II and III). It is important to remark that even when significant differences are found in the total amount of phenolics (values which may vary with the industrial treatment and cultivar used and with the maturity stage of the fruit), a single phenolic compound pattern is found.

Table II. HPLC Analysis of Fruit Jam Phenolics[a]

Apricot		
commercial sample	rutin	Km 3-rut
1	14.04	2.35
2	14.07	2.51
3	21.24	2.75
4	6.36	1.32
5	19.79	3.42
6	10.37	2.11
Peach		
commercial sample	Qu 3-glc	Km 3-glc
1	2.66	0.74
2	6.08	2.57
3	1.89	2.42
4	1.97	1.97
5	7.47	4.82
6	9.00	12.05
Plum		
commercial sample	caffeic	rutin
1	155.00	3.64
2	128.44	17.16
3	144.90	17.23
Strawberry			
commercial sample	Qu 3-glur	Km 7-glc	Km 3-glur
1	9.73	9.51	7.40
2	9.08	7.92	8.73
3	7.27	12.57	3.39

[a] Detection at 350 nm. Values are micrograms of phenolic per gram of jam (fresh weight). Km 3-rut, kaempferol 3-rutinoside; Qu 3-glc, quercetin 3-glucoside; Km 3-glc, kaempferol 3-glucoside; Qu-3-glur, quercetin 3-glucuronide + 3-glucoside; Km 7-glc, kaempferol 7-glucoside; Km-3-glur, kaempferol 3-glucuronide + 3-glucoside. All HPLC analyses were replicated, the mean values being reported. Reproducibility was ca. ±6%.

Table III. HPLC Analysis of Fruit Jam Phenolics[a]

Sour Orange			
commercial sample	neoeriocitrin	narangin	neohesperidin
1	80.00	115.98	95.00
2	51.28	79.48	64.10
3	56.41	96.15	84.61
Apple		
commercial sample	phloretin xylglc	phloridzin
1	1.73	2.32
2	5.18	9.16
3	0.42	1.00
Pear	
commercial sample	arbutin
1	113.33
2	37.17
3	6.53

[a] Detection at 280 nm. Values are micrograms of phenolic per gram of jam (fresh weight). All HPLC analyses were replicated, the mean values being reported. Reproducibility was ca. ±6%.

Identification of Phenolics in Fruit Jams. The different available commercial jams were extracted as described under Materials and Methods, and the major phenolic compounds were identified. Chlorogenic acid is

a common compound in all of the fruit jams analyzed as could be expected for such a widespread secondary metabolite (Figures 1 and 2).

The available apricot jams are characterized by the accumulation of rutin [quercetin 3-rhamnosyl(1–6)glucoside] and smaller amounts of kaempferol 3-rutinoside (Table II). These substances were identified by standard procedures and chomatographic comparisons with commercial authentic markers (rutin) and with kaempferol 3-rutinoside isolated from capers (Tomás and Ferreres, 1976). These compounds were identical with those reported previously from the natural apricot fruits (Henning and Herrmann, 1980a; Möller and Herrmann, 1983).

The analyzed peach jams generally contain a smaller amount of flavonoid compounds than apricot jams. This is probably due to the fact that the former are processed peeled, while the latter are processed unpeeled, and the flavonoid compounds accumulate in the skin (Herrmann, 1976). The available peach jams are characterized by the presence of quercetin 3-glucoside and kaempferol 3-glucoside (Table II). These results are in accordance with those found for different peach cultivars (Henning and Herrmann, 1980a). Both glycosides were identified by chromatographic comparisons (HPLC and TLC) with authentic markers. It is important to remark that quercetin 3-glucoside (characteristic compound of peach) and rutin (characteristic compound of apricot) show nearly the same retention time in the HPLC analyses with methanol–water mixtures on reversed-phase columns. Henning and Herrmann (1980a) clearly separated both compounds on straight-phase HPLC with silica gel columns, but acetylation of flavonol glycosides was necessary before analysis. In the present work we have applyed the Prisma solvent optimization system (Nyiredy et al., 1985) to the separation of rutin and quercetin 3-glucoside and found that using an isocratic system providing 25% solvent A (methanol–acetonitrile–tetrahydrofuran, 100:81:58) and 75% solvent B (water–formic acid, 95:5) with a flow rate of 1 mL/min on the reversed-phase column described under Materials and Methods, rutin eluted with a t_R of 5.0 min and quercetin 3-glucoside with a t_R of 6.2 min. These isocratic conditions are useful in studies of addition of apricot jam to peach jam (see below). In addition, these phenolics clearly differentiate by TLC on silica gel with 1-BuOH–HOAc–H_2O (4:1:5, upper phase), where rutin migrated with R_f = 0.45 and quercetin 3-glucoside with R_f = 0.58.

The plum jams analyzed show chromatograms in which caffeic acid is the main phenolic compound present, identified by chromatographic comparisons (HPLC) with an authentic sample. In additon, they contain significant amounts of rutin, which also was the main constituent of apricot jams, but differ from apricot since no kaempferol derivative was detected in significant amounts in plum jams. These results were in agreement with previous results on the phenolic compounds from plums (Henning and Herrmann, 1980b; Möller and Herrmann, 1983).

The available strawberry jams contain quercetin 3-glucuronide and kaempferol 3-glucuronide as major components, identified by their electrophoretic mobility on paper at pH 4.5, by chromatographic identification of the acid hydrolysis products (quercetin, kaempferol, and glucuronic acid), and by UV studies in methanol and after addition of classical shift reagents (Mabry et al., 1970), which clearly indicated that they were 3-glycosides of quercetin and kaempferol. In addition, they contain other not fully identified quercetin and kaempferol 3-glycosides and kaempferol 7-glucoside. This last compound was identified by its UV spectra in methanol and after addition of the classical shift reagents, which were characteristic of a kaempferol derivative with free hydroxyls at the C-3 (yellow fluorescence) and C-5 positions and substituted at the C-7 position (no shift on UV BII after addition of NaOAc (Mabry et al., 1970). After hydrolysis, kaempferol and glucose were identified. These results are in accordance with those reported for different strawberry cultivars (Ryan, 1971).

Sour orange jams contain a huge amount of flavonoids, by far the richest jams analyzed in the present work, including the bitter flavanone glycosides neohesperidin [hesperetin 7-rhamnosyl(1–2)glycoside], naringin [naringenin 7-rhamnosyl(1–2)glucoside], and neoeriocitrin [eriodictyol 7-rhamnosyl(1–2)glucoside] (Table III). These compounds were identified by chromatographic comparisons with authentic markers. In addition, a minor amount of the fully methylated flavonoid aglycons characteristic of sour orange such as sinensetin, nobiletin, and tangeretin had been detected and identified by chromatographic comparisons with authentic markers (Ferreres et al., 1980).

The available apple jams contain significant amounts of the dihydrochalcones phloretin 2′-glucoside (phloridzin) and phloretin 2′-xylosylglucoside, which are characteristic compounds from apple (Dick et al., 1987; Oleszek et al., 1988) (Table III), and they have in addition trace amounts of different quercetin glycosides. Phloridzin was isolated and identified by its UV spectra in methanol and after addition of the classical shift reagents and chromatographic comparisons with an authentic sample. Phloretin 2′-xylosylglucoside was also isolated, by a combination of paper chromatography and semipreparative HPLC, and identified by its UV spectra, which were identical to those of phloridzin, suggesting the same substitution pattern. After acid hydrolysis, the sugars glucose and xylose and the aglycon phloretin were identified by chromatographic comparisons (TLC, HPLC) with authentic markers. These results agree with those found for natural apple (Oleszek et al., 1988).

Pear jams contain an important amount of arbutin (1,4-dihydroxybenzene 1-*O*-β-D-glucoside) which was identified by enzymic hydrolysis with β-D-glucosidase which rendered 1,4-dihydroxybenzene, identified by chromatographic comparison with an authentic sample (HPLC) (Table III). In addition, minor amounts of isorhamnetin and apigenin glycosides were detected and their spectra recorded with the photodiode detector. The small amount of these substances prevented their full identification. However, these results were strongly in agreement with those found for different pear cultivars (Duggan, 1969; Spanos and Wrolstad, 1990).

These results show that every fruit jam contains one or more distinctive phenolic compound and a characteristic phenolic profile.

HPLC Phenolic Profile Analysis in the Determination of Authenticity of Fruit Jams. As a general rule, the phenolic compounds present in fruit jams coincide with those found in the corresponding natural fruits, and it is possible to know the characteristic phenolic compounds of every fruit and therefore the jams elaborated with them. The manufacturing process for obtaining jams does not affect the phenolic compounds pattern qualitatively, since the same profiles are observed for the different commercial jams originating from the same fruit. This industrial process might affect, however, the total content

of phenolic compounds as do the cultivar variety and the maturity stage of the fruit.

The extraction system described under Materials and Methods is useful for the purpose of the isolation and identification of the different phenolics from fruit jams. However, for screening purposes, an easier and quicker extraction technique should be used. For screening studies, 50 g of jam can be sonicated with 100 mL of 80% methanol for 15 min in an ultrasonic bath. The extracts filtered, concentrated under reduced pressure, and redissolved in acid water can be filtered through Amberlite XAD-2 as described under Materials and Methods. This extraction technique leads to the same phenolic compound profiles obtained with a more exhaustive extraction technique, although the total amount of phenolics extracted is smaller.

This technique could be used for the detection of mixtures of fruits in jams. This is especially important when cheaper fruits can be added to more expensive ones in a fraudulent manner. Thus, addition of apple to apricot, peach, or pear jams can be easily detected by determining the presence in the product of the characteristic compounds of apple, phloridzin and phloretin 2′-xylosylglucoside. This can be readily performed by HPLC analysis with detection at 280 nm, since at this wavelength no phenolic compound of apricot peach, or pear coincides in retention times with those of the characteristic dihydrochalcones of apple. The photodiode array detector, which records the characteristic UV spectra of the different phenolics, is the detector of choice for such a study.

Moreover, addition of apricot to peach jams, which is something possible when the harvest of peaches is not as good as expected and this fruit becomes more expensive, could be detected under the optimized HPLC isocratic conditions described above (Prisma), which clearly separate rutin from quercetin 3-glucoside. The presence of rutin, which is the main flavonoid of apricot, in peach jams could be considered evidence of apricot addition.

However, this technique has some limitations. For instance, the detection of addition of plum jam to apricot jam is not possible, since an increase in the amount of caffeic acid and rutin in the apricot jam chromatogram is not enough evidence to conclude that such a mixture has been made.

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