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COMPARATIVE STUDY OF THE POSSIBLE METHODS FOR THE QUANTITATIVE DETERMINATION OF PURE ORANGE CONTENT OF JUICES

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Abstract

Different methods (chemical analysis, comparison of UV spectra, SDS-PAGE and ELISA immunoassay) for determining the orange content of processed juices were studied. Characteristic differences were observed between fruit parts (albedo, flavedo, juice and pulp) and also between varieties in chemical composition (polyphenols, betaine, stigmasterin) and UV spectra. Thus it seems that these parameters may be used for the detection of non citrus additives and also of citrus by-products.

SDS-PAGE analysis of water-soluble proteins and the ELISA assay using antisera in rabbits give only tentative results due to the low specificity of antigens and the presence of other probably non protein immunoactive components.

Keywords: orange, orange juice, orange content in juices, ELISA, SDS-PAGE, chemical composition of, UV spectra.

1. Introduction

Due to the often increasing prices of oranges the processors introduce a lot of orange drinks containing cheaper components (sugar, citric acid, orange pulp, etc.) for dilution of the original juice. For the protection of customers, the development of reliable routine quality control measures providing information on the range of pure orange content in which a juice sample will be adequate, is becoming increasingly important. (KIMBALL; 1991, BELLIARDO and OOGHE, 1991; COCHET and DETAVERNIER, 1991). Complex chemical analysis of citrus composition results in valuable database of limited use in comparative studies, as the values depend on citrus variety, geographical conditions, analytical methods, etc. However, the complex analysis of citrus composition results in the detection of minor components (such as e.g. limonoid glucosides) (OZAKI et al., 1995; FONG et al., 1990; or flavour components (MOSHONAS and SHAW, 1986) which are diffi-

cult and uneconomical to forge, also in correlations among a large number of thoroughly tested parameters, which are characteristic of a certain citrus variety.

The determination of pure fruit content in drinks with low juice percentage (10-12%) is even more difficult. (AHMAD et al., 1986). Methods based on the high ion content characteristic of juices fail since ions could be added by water. Amino acids like serine (MORGAN, 1966) or betaine (LEWIS, 1966) are easily complemented. More complex tests such as aminoacid analysis (CLEMENTS, 1962) are too expensive. VANDERCOOK (1976) suggested a microbiological method for the determination of pure fruit content. As a simple and relatively inexpensive method, immunodiffusion was applied by FIRON et al. (1979), using pure orange juice as antigen. Antibodies were successfully used for a qualitative test for orange content determination. Limonin, the intensely bitter component of processed juices of citrus varieties was used as antigen in a sensitive, quantitative immunotest by POY (1984). Compared to other analytical separation methods (TLC, HPLC) the values for limonin content obtained by means of immunobiochemical methods were found more reliable by CARTER et al. (1985).

In a collaborative study, the real values for limonin content were met with 94% and 111% precision by EIA or RIA, respectively, while TLC and HPLC methods resulted in only 53–70% and 65–100% accuracy. EIA methods using endogeneous cytokinins (zeatin riboside and isopentenyl adenosine) of citrus varieties as haptens conjugated to bovine serum albumin was described by BARTHE et al., (1985). The detectability is reported to be 50 pg of cytokinins.

The present work focuses on the reliability of different analytical methods for estimating the pure orange content of commercial drinks using three different techniques: complex chemical analysis of citrus compounds (mediterranean-Greek and tropical-Cuban) orange and mediterranean (Cyprus) lemon, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and Enzyme Linked Immuno Sorbent Assay (ELISA).This was a collaborative study involving two separate laboratories.

2. Materials and Methods

2.1 Materials

Samples of 15 commercial orange concentrates from different Hungarian processing factories, declared to be of pure orange, and three controlled juices prepared from pure oranges (grown in Greece and Cuba) using the same technology as the factories, were provided by the Analytical Chemical Department of the Central Food Research Institute, Budapest, Hun-

gary. The yellow (flavedo) and white (albedo) parts of the orange peel, the pressed juice and the pulp of two orange cultivars (grown in Greece and Cuba) as well as lemon juice were also supplied. Pressed juice was obtained by vacuum filtering the juice of pressed orange halves through sintered glass funnel. As pulp, the precipitate of peeled and blended oranges was used after centrifugation at 3000 g for 10 min.

2.2 Methods

2.2.1 Chemical analysis and UV spectra of citrus compounds

The nitrogen content, the betaine content, the total polyphenol concentration, the stigmasterine content and the lipid content of the different morphological parts of two orange cultivars and lemon were determined by means of Hungarian standard methods.(LÁSZTITY and TÖRLEY, 1987). Total amino acid content was measured by using a Kjelfoss apparatus, calculated from the nitrogen content. UV spectra of the polyphenols occurring in the samples were recorded in the range of 240–400 nm using a Beckman Model 165 UV detector (Beckman Instruments, Inc., Fullerton, USA).

2.2.2 SDS-polyacrylamide electrophoresis

Water soluble proteins in the samples were separated and their molecular weights were determined by SDS vertical slab electrophoresis according to WEBER and OSBORNE (1969). Proteins were denatured in the presence of urea and stained with the silver nitrate method (MERRIL et al., 1982). Slab gels were evaluated with a Shimadzu Model CS-930 dual-wavelength thin-layer chromato-scanner.

2.2.3 Antisera

Water soluble protein from 1 ml orange samples after ammonium sulphate precipitation and dialysis against distilled water was used as antigen. Antisera were raised in rabbits inoculated every two weeks by the protocol described by JOURDAN et al. (1983). Serum was collected after twelve weeks and stored at 10 °C. It was purified following ammonium sulphate precipitation according to STEINBUCH and AUDRAN (1969).

2.2.4 ELISA immunoassay

Sample aliquots of 100 μl volume were applied in 50× and 100× dilutions to microtiter plates using 40 mmol/l $NaHCO_3$ at pH 9.6. After overnight

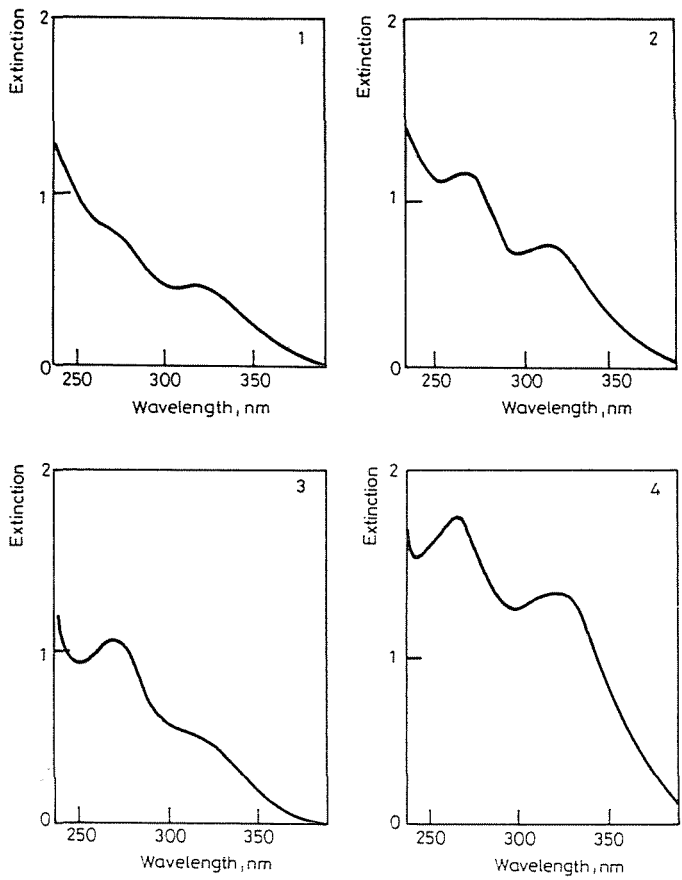

Fig. 1. UV-spectra of samples produced from Greek orange 1 – pressed, filtered juice, 2 – pulp, 3 – albedo, 4 – flavedo.

incubation at 4 °C, the solution was discarded and the wells were washed with distilled water. To saturate the uncoated adsorption sites, the wells were incubated at RT for 60 min with 0.01% solution of BSA in Tris-HCl buffered saline (TBS), and washed as before. To each antigen coated well was added a 5 μg/ml solution of anti-orange IgG (200 μg/well). The plate was sealed and incubated at 24 °C for 2 h. The liquid was then decanted and the wells were rinsed several times with PRS. To each well, goat anti-rabbit IgG, labelled with peroxidase, was added in 500× dilution and the plate was incubated for another hour at 37 °C. Excess reagents were rinsed off and washed with PBS containing 0.05% Tween 20. For the detection of POX activity, 100 μl of substrate (1 mg/ml of *o*-phenylene-diamine) was

added to each well and the plate was incubated for 10 min at 38 °C. The substrate reaction was stopped by addition of 50 μl of 1 mol/l H_2SO_4 and the plate was read at 405 nm.

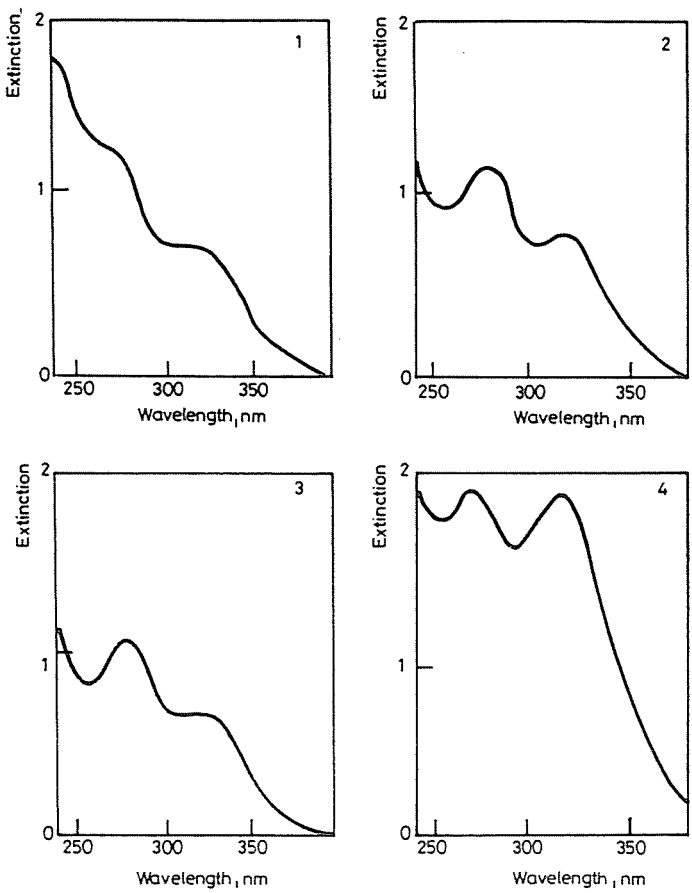

Fig. 2. UV-spectra of samples produced from Cuban orange 1 – pressed, filtered juice, 2 – pulp, 3 – albedo, 4 – flavedo.

3. Results and Discussion

3.1 Chemical Analysis and UV Spectra of Citrus Compounds

The chemical composition varied for the different morphological parts of fruits. The nitrogen content shows increasing tendency in the order of juice,

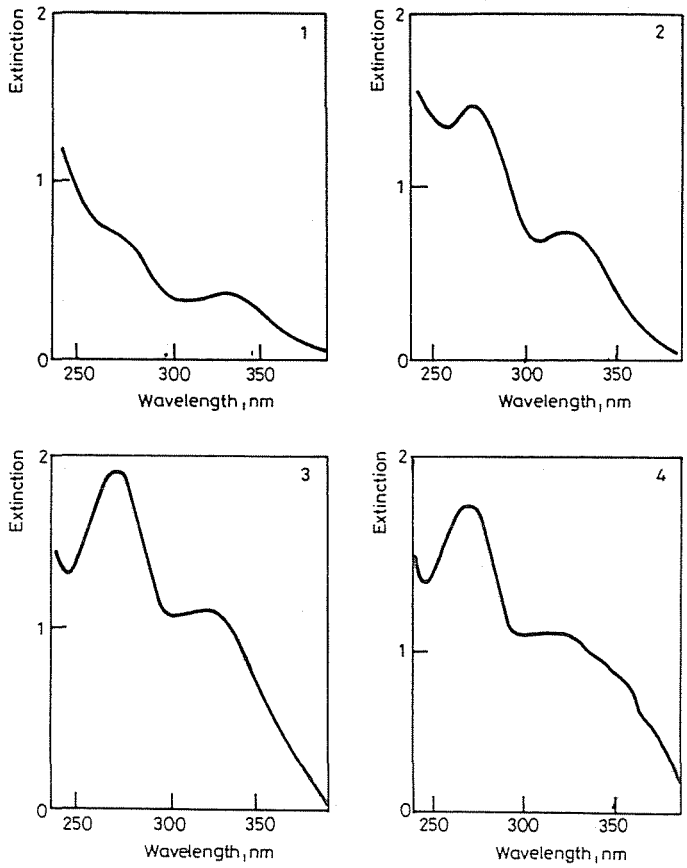

Fig. 3. UV-spectra of samples produced from lemon 1 – pressed, filtered juice, 2 – pulp, 3 – albedo, 4 – flavedo.

pulp, albedo and flavedo (*Table 1*). The total amino acids content shows similar tendency. However, the betaine content is accumulated mostly in the juice and the pulp. Of the investigated nitrogen compounds the phenolic amines were detected by thin-layer chromatography (data not shown). For quality control this group is one of the most important as several amines such as simpatomine and tyramine are characteristic of a citrus variety. The polyphenol concentration increases towards the peel, being accumulated in the flavedo. In all samples, the stigmasterine is concentrated in the pulp and albedo. The lipid content varies with the fruit, which helps in detecting the presence of lipid additives of non-citrus origin.

Table 1
The nitrogen, polyphenol, stigmasterine, volatile oil and lipid content of Greek orange, Cuban orange and lemon juice samples

Sample	Nitrogen content g/100g	Betaine HCl mg/100g	Total amino acid meq/100ml	Total polyphenol E/100 ml	Total amino acid/total polyphenol	Stigmasterine mg/100g	$CHCl_3$/MeOH soluble %
Greek orange							
Juice	0.48	576	1.52	0.76	2.00	0	0.53
Pulp	0.54	577	1.93	1.46	1.32	44	1.08
Albedo	0.40	165	1.71	1.68	1.02	58	3.05
Flavedo	0.70	173	3.23	8.76	0.37	14	0.00
Cuban orange							
Juice	0.49	423	1.74	0.76	2.30	24	0.20
Pulp	0.55	1320	2.04	1.60	1.28	133	0.31
Albedo	0.78	762	3.33	5.26	0.63	58	0.00
Flavedo	0.78	260	3.10	14.96	0.21	27	1.28
Lemon							
Juice	0.32	408	2.74	0.64	4.30	51	0.22
Pulp	0.49	467	3.51	1.42	2.47	163	0.19
Albedo	0.80	25	2.72	3.37	0.81	44	1.24
Flavedo	0.72	0	5.22	8.06	0.65	24	0.62

The UV spectra of the morphological parts of the investigated orange cultivars and lemon sample show characteristic differences (*Figs. 1, 2* and *3*). The spectra of albedo and the pulp from Greek and Cuban oranges are quite similar. However, the flavedo curves of the two oranges are different. Similarities may be observed between the flavedo of lemon and Greek orange. Also the spectra of juices from lemon and Greek orange are similar. The highest polyphenol content was found in the juice of Cuban orange. The lemon pulp and albedo are the richest in polyphenols. The data of UV spectra support the results of chemical analysis (data shown in *Table 1*).

In summary it could be concluded that the differences in chemical composition and UV spectra of different parts (albedo, flavedo, pressed juice, pulp) of the investigated fruits suggest a possibility of detection of citrus by-products and non-citrus additives in orange concentrates.

3.2 SDS-PAGE of Proteins

Purification of the water soluble proteins by precipitation with ammonium sulphate and subsequent dialysis allowed a good separation of proteins.

Table 2
Calculated amounts of pure orange content of some commercial juice samples based on the immunoassay values

Nr.	Sample	Calculated amount of pure orange juice content (%) Determined by ELISA applying 50× dilution	100× dilution
1	Orange concentrate	96.2±2.5	99.8 ±12.6
2	Orange concentrate	114.1±4.1	123.4±2.8
3	Orange concentrate	95.8±2.3	87.0 ±4.2
4	Orange concentrate	67.6±6.3	103.8±8.6
5	Orange concentrate	90.1±2.7	81.8 ±5.1
6	Orange concentrate	80.3±1.8	125.9±20.1
7	Orange concentrate	90.1±5.1	136.3±11.2
8	Orange concentrate	56.3±1.7	101.3±15.8
9	Orange concentrate	70.4±2.1	71.4 ±1.6
10	Orange concentrate	100.0±6.5	114.2±5.5
11	Orange concentrate	91.9±1.2	76.6 ±3.2
12	Orange concentrate	89.4±1.3	75.3 ±2.1
13	Orange concentrate	116.2±0.5	94.8 ±1.3
14	Orange concentrate	114.2±1.3	103.8±2.7
15	Orange concentrate	89.4±2.1	90.9 ±1.8
16	Control pure orange juice	100.0	100.0
17	Albedo	53.5±3.3	63.6 ±7.1
18	Flavedo	51.7±1.4	56.5 ±1.3
19	Pressed filtered juice	36.6±1.2	41.6 ±2.7
20	Pulp	22.2±1.1	30.1 ±3.3
21	Pure fresh lemon juice	60.8±4.2	50.0 ±5.5

High similarity was found for samples 1 through 9, 10, 11, 13. Similar observations could be made in relation to albedo, flavedo, and pulp samples of the investigated oranges. A lower degree of similarity is characteristic of the pressed juices. A double band in the region of 12–14 kD was observed in all orange products and morphological parts of orange. The lemon juice appeared to be characteristically different with double bands in the higher molecular weight range. Some typical electropherograms are shown in *Fig. 4*.

3.3 ELISA Analysis

In *Table 2* characteristic results based on the ELISA absorbancy are collected. The average value of the three controlled orange juices was used as the control value (100%). As seen, the majority of the commercial samples showed values being near the control value, only samples 4, 8, and 9

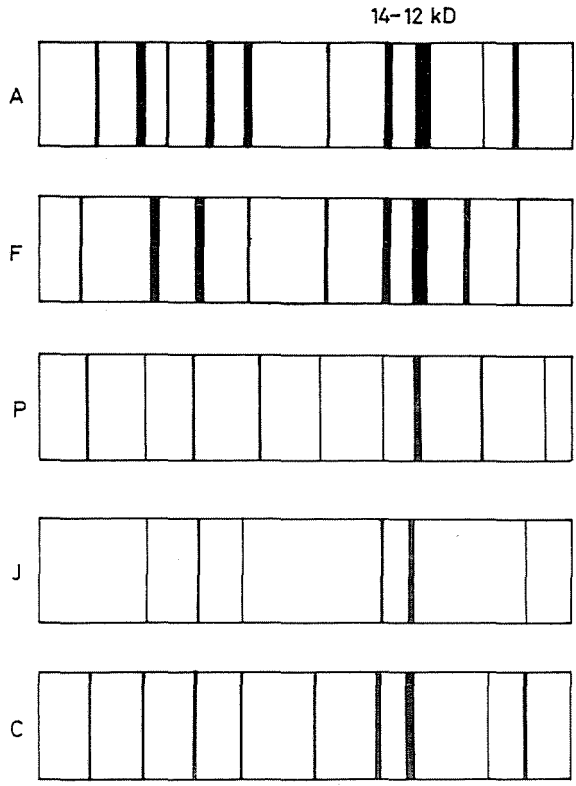

Fig. 4. Characteristic PAGE electropherogram schemes of samples A – albedo, F – flavedo, J – pressed, filtered juice C – orange juice concentrate

had quite different absorbancy data. In some cases differences were observed between the data obtained using 50× dilution and those with 100× dilution. This may be probably explained by assuming that the increased amount of alkaline buffer used for dilution may enhance the adsorption of orange sample antigens.

The immune reaction of lemon proteins with anti-orange IgG shows that the antibody is not specific for orange. Immune reactions of albedo, flavedo and pulp exclude the possibility of quantitating the pure orange content of high quality drinks. Some values over 100% suggest that there could be present immunoactive components than the orange proteins.

As conclusion it could be stated that the ELISA method based on antibodies of orange proteins is not suitable for controlling the orange content of drinks. The eventuell use in the detection of non-citrus components needs further investigations.

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