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Fibre fraction carbohydrates in *Olea europaea* (Gordal and Manzanilla var.)†

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Olive dietary fibre, Gordal and Manzanilla varieties, has been isolated by chemical (acid and neutral detergent fibre of Van Soest) and enzymatic (soluble and insoluble fibre of Asp) methods. NDF and ADF values were lower than IF. All of the fractions were hydrolysed and the resultant neutral sugars quantified. Glucose was the main component in NDF, ADF and IF, and arabinose was the major one in SF. The content of protein was significantly different between fractions but not between varieties, and the content of cellulose was significantly different between fractions and varieties. The determination of hemicelluloses by the Van Soest method showed a very low precision (cv. >40%); determined by acid hydrolysis there were significant differences between fractions and varieties.

INTRODUCTION

Nowadays there is ample evidence that the lack of fibral components, combined with growing consumption of sugar, can seriously affect man's health as he finds himself increasingly susceptible to what have been labelled 'diseases of Western Civilization'. This fact has given rise to the situation in which the study of fibre is considered implicit in any type of study related to vegetable nutrition because of its capacity for acting as a preventive agent in cardiovascular diseases and cancer of the colon.

There is an extensive bibliography about the methods of fibre analysis and its components (Van Soest & Wine, 1968; Hellendoorn *et al.*, 1975; Schweizer & Wursch, 1979; Selvendran & Dupont, 1980; Furda, 1981; Southgate, 1981; Englyst *et al.*, 1982; Asp *et al.*, 1983; Prosky *et al.*, 1984; Mongeau & Brassard, 1986; Theander & Westerlund, 1986). However, not all the methods applicable to other vegetable products are appropriate for the olive, as a consequence of its peculiarities, fundamentally due to its high oil and polyphenols contents.

The olive is a fruit of great importance, both because of its oil and for the organoleptic characteristics and nutritive value of the varieties used as table olives (dressed fruit) (Castro *et al.*, 1979). In both cases, the characteristics of the fresh fruit, that later has to be prepared, turn out to be of great interest. Fibre is the one component of this fruit that determines its texture and digestibility. As there is no universal method for fibre, it is necessary to select the most suitable one for each vegetable product. Present research involves a comparative study of several systems for the isolation of fibre and the analysis of the sugars which are obtained through its hydrolysis, using fresh olives of the Gordal and Manzanilla varieties; this is carried out as a preliminary step before moving on to study the modifications that olives go through as a result of the dressing process.

The methods chosen have been the detergent system of Van Soest and the enzymatic one of Asp. Both are extensively used and they represent two different ways for fibre preparation: the chemical and the enzymatic.

MATERIALS AND METHODS

Materials

Olives of Manzanilla and Gordal varieties were harvested in the Province of Seville (Spain). Sampling was carried out on three trees, harvesting the fruits all around, inside and outside, from the upper and lower part of each one of them, in order to get a 500 g sample, which

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might be considered as representative enough of the average maturity stage. The fruits were stored at 4°C for no longer than 24 h until used. All reagents were of analytical grade.

Moisture

The olive samples were de-stoned and the flesh triturated to a homogeneous (if possible) paste. A sample of 5 g paste was weighed to 0·1 mg, in a tared container, and dried at 60–70°C to constant weight, with a partial vacuum of about 25 mm.

Fat extraction

The dry flesh was extracted (Soxhlet) with hexane (b.p. 60–70°C) for 5 h. The solvent was separated in a rotary evaporator at 40°C and the oil dried in the oven at 100°C.

Protein determination

The proteins were estimated by the micro Kjeldahl method (0·1 mg/ml detection limit). Kjeldahl nitrogen was converted to protein by use of the 6·25 factor.

Free sugars

These were determined by high pressure liquid chromatography (HPLC). The chromatographic separation was carried out on an Aminex HPX 87 carbohydrate column (BioRad Labs, Richmond, California, USA), using water as mobile phase. The flow rate was 0·9 ml/min under isothermic conditions at 85°C. Ethanol (96%) (250 ml) was added to olive flesh (50 g), and reflux-heated for 2 h, left to cool and filtered through a Büchner funnel. The filtrate was concentrated in a rotary evaporator to dryness (30°C), distilled water was added to dissolve the sugars, and the mixture was filtered. Filtration was repeated using filter paper and the solution made up to 500 ml in a standard flask. The solution was covered with a thin layer of toluene to prevent microbial development, and kept in the refrigerator. 2-Deoxyglucose solution (1%) (2 ml), used as internal standard, was added to the sugar solution (2 ml), which was passed through ion-exchange resins. The columns were washed with water (30 ml for each column) and the effluent was evaporated to dryness in a rotary evaporator (30°C). Distilled water (2 ml) was added and the resulting extract was filtered using a Millipore filter (0·25 μm) and passed through a Sep-pack C18 (Waters Associates, Milford, Massachusetts, USA) in order to eliminate pigments and polyphenols.

Fibre determination

Neutral detergent fibre and acid detergent fibre were determined from the de-fatted flesh, using the procedure of Van Soest (1963*a*,*b*). Insoluble and soluble fibre were isolated by the method of ASP *et al.* (1983).

Acid hydrolysis

The polysaccharides of ADF, NDF, IF and SF were subjected to sequential hydrolysis with concentrated sulphuric acid and then more dilute acid. Sulphuric acid (72%) (1 ml) was added to 250 mg of sample, which was placed in a thermostatic bath at 40°C and stirred for 2 h. Next, water (11 ml) was added, resulting in a solution of 2 N sulphuric acid, and the heating continued at 100°C for 2 h. After neutralizing with NH_4OH, the sugars in the solution were identified and quantified.

Neutral sugars determination

The analysis of the sugars resulting from hydrolysis was carried out by GC. The alditol acetates were prepared by the method of Englyst *et al.* (1982) with few modifications. Myoinositol water solution (4 mg/2 ml) as internal standard was added to the sugar solution which was filtered. Ammonia solution, (110 μl) 12 M, and 0·1 ml of sodium borohydride (100 mg/ml) were added to 1 ml of the solution, and the mixture was left to settle for 1 h at 40°C. The excess reagent was eliminated with concentrated acetic acid until bubbling stopped. 1-Methylimidazole (0·3 ml), acting as catalyst, and acetic anhydride (2 ml) were added to 100 μl of this solution. After 5 min, water (5 ml) and dichloromethane (1 ml) were added. The aqueous phase was discarded and the organic phase evaporated to dryness in a rotary evaporator (30°C). The alditols were dissolved in chloroform (0·1 ml) and injected. A capillary column of fused silica (30 m × 0·53 mm) was used, with SPTM 2380 of Supelco as liquid phase. A Perkin Elmer chromatograph, model 3920B, equipped with flame ionization detector was used for the determinations. Nitrogen was used as the carrier gas with a flow rate of 3 ml/min. The auxiliary gas flow was 60 ml/min, the detector temperature was 300°C, and that of the column 250°C. The volume of sample injected was 2 μl.

Statistical analysis

The data obtained were statistically treated and the mean standard deviation calculated. Coefficients of variation for the analytical error of the sugar methods were calculated by analysis of variance. The comparison of means has been done according to the Duncan's new multiple range test.

RESULTS AND DISCUSSION

Fruit composition

The fruit had been picked in the best conditions of ripeness for pickling (ripe green fruit) and the determi-

Table 1. Composition of olives (*Olea europaea*, var. Gordal and Manzanilla)

	Composition (%)	
	Gordal	Manzanilla
Moisture	71·62 ± 0·06	69·66 ± 0·04
Dry matter	28·38	30·34
Fat	15·19 ± 0·30[a]	16·03 ± 0·44[a]
	53·52[b]	50·77[b]
Protein	1·29 ± 0·14	1·20 ± 0·07
	4·53	3·95
Free sugars	3·94 ± 0·55	3·75 ± 0·54
	13·88	12·36
Ash	0·01 ± 0·007	0·01 ± 0·006
	0·58	0·47
Free sugars		
Sucrose	0·30 ± 0·03	Traces
	1·04	
Glucose	2·97 ± 0·35	2·33 ± 0·23
	10·46	7·68
Fructose	0·37 ± 0·03	0·90 ± 0·17
	1·30	2·95
Mannitol	0·31 ± 0·07	0·37 ± 0·00
	1·07	1·22

[a] Fresh flesh.
[b] Dry matter.
All values are means of four analyses ± standard deviation.
The olive does not contain starch (Heredia, 1976).

nations made on the varieties Gordal and Manzanilla. The results obtained for moisture, fat, proteins and free sugars are shown in Table 1. The determination of free sugars was carried out by HPLC: the content varied between 3 and 4% of fresh flesh, and among them were detected glucose, mannitol, sucrose and fructose, glucose being the major one.

Fibre determination

The chemical method of Van Soest & Wine (1968) and the enzymatic one of Asp (1983) were used. The former determines the neutral detergent fibre and the acid detergent fibre (NDF and ADF), and the enzymatic method permits an evaluation of the total fibre (TF), the sum of the soluble (SF) and insoluble (IF). Table 2 summarizes the results of NDF, ADF, SF and IF for both varieties. The major one is IF, with very similar values for Gordal and Manzanilla. The percentages of SF are close to 1%, and those of TF close to 5%, representing a high proportion of this fruit. The fibre values obtained by the system of Van Soest (NDF and ADF) are lower than those of IF and TF.

Lund *et al.* (1983), studying the fibre of certain tropical fruits, also obtained higher values of IF, attributing this to the presence of pectins, which are, however, eliminated in the NDF treatment. Schweizer and Wursch (1979) found a similar effect on analysing a series of cereals, vegetables, and fruit, explaining that the differences could be due to the insoluble pectins and other polysaccharides. Dudek *et al.* (1985) found in pears that IF is approximately equal to NDF plus pectins. Another important factor which explains the differences between NDF and IF is the presence of condensation products of phenolic compounds with polysaccharides and proteins in polyphenol-rich vegetables, as is the case for the olive, which may contain more than 5% of such products (Vázquez *et al.*, 1971). This fact coincides with that found in sainfoin (Saura-Calixto, 1987). Thus it can be concluded that the NDF method is much more efficient in solubilization of the condensed phenols (Reed *et al.*, 1982).

Table 2. Fibre fractions, Gordal and Manzanilla var.

	Fibre Fractions (%)	
	Gordal	Manzanilla
ADF	2·08 ± 0·08[a]	2·41 ± 0·08[a]
	7·32[b]	7·95[b]
NDF	2·83 ± 0·23	3·19 ± 0·10
	9·96	10·52
IF	4·03 ± 0·09	4·26 ± 0·15
	14·17	14·05
SF	1·01 ± 0·13	0·65 ± 0·32
	3·55	2·15
TF	5·04	4·91
	17·72	16·20

[a] Fresh flesh.
[b] Dry matter.
All values are means of four analyses ± standard deviation.

Acid hydrolysis of the fibre fractions

After carrying out a study of the optimum conditions, a primary hydrolysis was established with H_2SO_4 (72%) for 2 h at 40°C, and a secondary hydrolysis with H_2SO_4 (2 N) for 2 h at 40°C. Table 3 shows the composition of neutral sugars. Glucose is the major sugar in all the fractions except for SF, is which it is arabinose. The highest percentages of galactose are found in IF, whereas this sugar is partially solubilized in NDF, and only traces appear in ADF. The mannose content is notably equal in IF, ADF and NDF, and much lower in SF. A great part of the xylose is solubilized in the ADF treatment, only low percentages appear in SF, while the highest percentages are those in IF and NDF. An important fraction of arabinose is lost in the NDF treatment, practically disappears in the ADF; it is found in high percentage in IF and much lower in SF. Rhamnose is present in small quantities in all the fractions.

Proteins

Table 4 shows the protein values of the original flesh and each fibre fraction, as well as the non-solubilized

Table 3. Hydrolysate composition of the fibre fractions

	Hydrolysate composition (%)			
	IF	SF	ADF	NDF
Gordal				
Glucose	1·04	0·08	1·12	0·610
Galactose	0·03	0·03	0·001	0·012
Mannose	0·03	0·004	0·030	0·020
Xylose	0·27	0·018	0·087	0·120
Arabinose	0·24	0·120	0·004	0·073
Rhamnose	0·02	0·015	Traces	0·004
Manzanilla				
Glucose	1·08	0·011	0·560	0·543
Galactose	0·077	0·014	0·003	0·016
Mannose	0·028	0·001	0·018	0·018
Xylose	0·128	0·001	0·046	0·103
Arabinose	0·171	0·016	0·008	0·097
Rhamnose	0·004	0·003	Traces	0·008

Percentages referred to fresh flesh.
All values are means of two determinations (cv. 10%).

Table 4. Fibre proteins (%) in Gordal and Manzanilla varieties

	Gordal		Manzanilla	
	Protein	Non-solubilized protein (%)	Protein	Non-solubilized protein (%)
Fresh flesh	1·27 ± 0·14	—	1·20 ± 0·07	—
ADF	0·07 ± 0·01	5·42	0·39 ± 0·02	32·50
NDF	0·48 ± 0·01	37·2	0·47 ± 0·03	39·17
IF	0·73 ± 0·14	56·6	0·79 ± 0·06	65·80

Percentages referred to fresh flesh.
All values are means of four analyses ± standard deviation.

proteins of each. According to the analysis of variance, referred to NDF, ADF and IF, significant differences between fractions, but not between varieties were observed.

With respect to the non-solubilized proteins, the highest values are those of IF, in which between 56 and 65% of the original proteins remain, approximately 40% in NDF, and the percentages are very different in ADF for both varieties (5% in Gordal and 32% in Manzanilla). The presence of proteins in the fibre fractions could be due to:

(a) Formation of Maillard compounds (products of condensation of proteins and carbohydrates) as a consequence of the heating process. These compounds are insoluble, and thus could appear in the fibre fraction.
(b) Formation of products of condensation between tannins and proteins.
(c) Enzymatic inhibition of the proteases used in the digestion by the polyphenols present (Robertson & Van Soest, 1981; Saura-Calixto, 1987).

In the case of the olive, (b) and (c) could have an important influence and be responsible for the high percentages of proteins which appear. The difference existing between the protein contents of NDF and ADF is a fact which has also been observed in other products and which contributes to an over-evaluation of hemicelluloses. From the point of view of the estimation of the indigestible residue, there is no problem in the fact that proteins appear in the fibre components (Dreher, 1987). In some products, such as wheat bran, good correlation has been obtained between the content of NDF fraction proteins consumed and excreted by individuals fed with wheat bran (Saunders & Betschart, 1980; Dintzins, 1982).

Cellulose

Cellulose determination was carried out: (a) as weight loss on incinerating the de-lignified ADF fraction; (b) by acid hydrolysis of each fibre fraction, quantifying the cellulose as glucose.

The results obtained are shown in Table 5. Significant differences between fractions and varieties were observed. The higher values are obtained by Van Soest's method and this could be explained by the fact that sugars corresponding to hemicelluloses have been detected in the de-lignified ADF fraction (Table 6).

Hemicelluloses

These were determined: (a) as the difference between the NDF and ADF fractions (Van Soest's method); (b) by acid hydrolysis of each of the fibre fractions, considering all the sugars, except glucose, as hemicellulose components. The results (Table 5) showed that very low precision was achieved with the Van Soest method (cv. > 40%). In relation to the hemicellulose content of ADF, NDF and IF, the highest value was found for IF. It is important to note that a significant quantity of hemicellulose was present in ADF fractions. Significant differences between varieties were observed.

As a result of this work the following conclusions can be drawn:

1. The enzymatic digestibility of proteins is very low as indicated by the high protein content of IF.
2. The cellulose content of the NDF fraction is lower than those of ADF and IF; it seems as if there is a loss of cellulose during NDF treatment and even in the ADF treatment in the Manzanilla variety.
3. The system of Van Soest for estimation of hemicelluloses as difference between NDF and ADF does not seem suitable for this product.
4. Adding the values of cellulose, hemicelluloses, proteins and lignin for each fibre fraction (Table 5), it can be seen that between 20 and 25% of IF and NDF remains undetermined; this fraction may be constituted by pectin and condensed phenols and it will be the subject of future research.

Table 5. Fibre fraction composition

	Fibre fraction composition (%)		
	IF	ADF	NDF
Gordal			
Cellulose	1·05 ± 0·008	1·12 ± 0·11 (1·24 ± 0·08)[a]	0·60 ± 0·02
Hemicellulose	0·60 ± 0·04	0·12 ± 0·004	0·23 ± 0·0008 (0·75 ± 0·28)[a]
Lignin	0·83 ± 0·008	0·83 ± 0·007	0·83 ± 0·0001
Protein	0·73 ± 0·004	0·07 ± 0·001	0·48 ± 0·002
Ash	0·06 ± 0·002	0·01 ± 0·003	
Manzanilla			
Cellulose	0·81 ± 0·04	0·56 ± 0·03 (1·14 ± 0·08)[a]	0·54 ± 0·07
Hemicellulose	0·36 ± 0·01	0·07 ± 0·0007	0·24 ± 0·03 (0·77 ± 0·34)[a]
Lignin	1·16 ± 0·01	1·16 ± 0·01	1·16 ± 0·01
Protein	0·79 ± 0·06	0·39 ± 0·004	0·47 ± 0·007
Ash	0·12 ± 0·01	0·01 ± 0·006	

[a] Determined by Van Soest method.
Percentages referred to fresh flesh.
All values are means of four analyses ± standard deviation.

Table 6. Hydrolysate composition of the ADF (original and de-lignified) (Gordal and Manzanilla varieties)

	Gordal		Manzanilla	
	Original	De-lignified	Original	De-lignified
Glucose	1.22	0.820	0.560	0.536
Galactose	0.001	0.001	0.003	0.006
Mannose	0.030	0.018	0.018	0.017
Xylose	0.087	0.052	0.046	0.025
Arabinose	0.004	0.003	0.008	0.001
Rhamnose	Traces	Traces	Traces	Traces

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