Grasas y Aceites
Vol. 47. Fasc. 4 (1996), 247-254

Natural antioxidants of virgin olive oil obtained by two and tri-phase centrifugal decanters

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RESUMEN

Antioxidantes naturales de aceite de oliva virgen obtenido por decantadores centrífugos de dos y tres fases.

El nuevo decanter de dos fases no requiere adición de agua al proceso de pasta de aceituna y produce cantidades muy limitadas de agua de vegetación. Por consiguiente, la eliminación del enorme problema de las aguas residuales hace particularmente interesante esta innovación tecnológica. Por tanto, en orden a evaluar, en comparación con el decanter tradicional, su efectividad en relación con la composición cuali-cuantitativa de antioxidantes naturales, tocoferoles y compuestos fenólicos se determinaron sobre muestras de aceites de oliva vírgenes obtenidos por decantadores centrífugos de dos y tres fases. Particularmente, las sustancias fenólicas se analizaron por métodos colorimétricos, HPLC y HRGC.

Los resultados obtenidos provaron que los aceites extraídos por decanter de dos fases tuvieran concentraciones más altas de tocoferoles y fenoles, especialmente o-difenoles y mostraron una estabilidad a la oxidación también más alta.

La resistencia a la oxidación presentó mejor correlación con el contenido de o-difenoles simples y ligados y de la aglicona de decarbometoxiligustrósido.

PALABRAS-CLAVE: Aceite de oliva virgen – Antioxidante natural – Autoxidación (estabilidad) – Decanter centrífugo de dos fases – Decanter centrífugo de tres fases.

SUMMARY

Natural antioxidants of virgin olive oil obtained by two and tri-phase centrifugal decanters.

The new dual-phase decanter does not require any addition of water to process olive paste and produces very limitated quantities of vegetable water. Consequently, the elimination of big waste water problem makes particularly interesting this technological innovation. Therefore, in order to evaluate, in comparison with the traditional decanter, its effectiveness in relation to the quali-quantitative composition of natural antioxidants, tocopherols and phenolic compounds were determined on samples of virgin olive oils obtained by centrifugal decanters at two and three phases. Particularly, phenolic substances were analysed by colorimetric, HPLC and HRGC methods.

The results obtained proved that oils extracted by dual-phase decanter had higher concentrations of both tocopherols and phenols, especially o-diphenols, and showed higher stability to oxidation.

The resistence to oxidation appeared better correlated with the content of simple and linked o-diphenols and of aglycon of decarbomethoxy ligstroside.

KEY-WORDS: Autoxidation (stability) – Natural antioxidant – Tri phase centrifugal decanter – Two phase centrifugal decanter – Virgin olive oil.

1. INTRODUCTION

Between 1970s and 1980s centrifugation system for extracting oil from olive pastes is widely spread in oil industry because of its indisputable advantages, that is cutting of processing costs and shortening of fruit storage time before the extraction, with undeniable improvement of oil quality, particularly in southern regions of Italy (Cucurachi, 1975).

However, the need of warm water addition to dilute olive paste, before it goes in to the centrifugal decanter, has as inevitable consequences both the lowering of natural antioxidant compounds in resulting oils, because of their higher solubility in aqueous phase (De Felice et al., 1979; Di Giovacchino et al., 1980; Di Giovacchino and Solinas, 1992), and the considerable increase of volume of vegetable water produced by the processing plant, which aggravates waste disposal problems and costs.

Attempts to obviate these troubles are made recycling the vegetable water as soon as it is produced and using it instead of ordinary water for dilution of pastes. The findings obtained on applying this technique evidence a 35-40% loss in the volume of waste water and an increase of about 30% in polyphenol content of the oil (Amirante et al., 1992).

Recently, some olive oil plant manufactures threw on the market new models of decanter, able to separate the oily phase from the malaxed olive paste without requiring any addition of warm water; furthermore the utilization of these decanters produces very limited quantities of vegetable water (10-20 l/100 olive Kg) or does not involve any its production, since it remains in the pomace, which is therefore moister.

The elimination of the big waste water problem makes particularly interesting this technological innovation and therefore at once, in a previous research (Di Giovacchino, 1994), the aspects connected with quantity oil yields, by-product characteristics and oil production quality, in comparison with conventional centrifugation system, were checked.

The results obtained evidenced satisfactory extraction yields, slightly moister pomaces, very low costs for waste disposal; moreover, the findings showed that oils extracted by dual-phase decanter had higher content of total polyphenols and of o-diphenols and, consequently, higher resistence to oxidation.

It is very well known that polyphenols are tightly connected to oil stability to autoxidation because of their prevention action of the production and accumulation of peroxydes (Montedoro, 1972; Walter et al., 1973; Gutfinger, 1981; Papadopoulos and Boskou, 1991).

The constant higher level of polyphenols in oils extracted by dual-phase decanter induced us to evaluate more carefully the incidence of decanter kind on the quali-quantitative composition of phenolic fraction and to evidence, if possible, any correlations with oil stability.

2. EXPERIMENTAL PART

During the 93-94 campaign, olive oil extraction trials were performed with a dual-phase decanter and with a conventional tri-phase direct centrifugation plant. With this aim, a Novoil S 1 plant with a 4000-S Ecologico decanter, manufactured by Rapanelli company of Foligno, was used; the decanter was adjusted to work on three phases (with addition of water to olive paste) or on dual-phase (without water addition).

Olives of different varieties, cultivated in Abruzzo and harvested at various ripening degree, were used in the trials.

The operating conditions are the following: olives deleafed were crushed with a moving hammer crusher, and the paste was malaxed for 60 minutes at 22°C; only when plant worked on three phases, 60-80 l/100 olive kg of water was added to olive paste, and then oily must was separated in an upright automatic-discarge centrifugal separator.

On the resulting oils were performed the following determinations:

– Evaluation of organoleptic characteristics, free acidity and peroxide value according to EC Regulation n. 2568/91.

– Rancimat stability (Laubli and Bruttel, 1986);

– HPLC analysis of tocopherols on silica column, using as mobile phase hexane –2– propanol and UV detection at 295 nm (Angerosa and Marsilio, 1983);

– HPLC analysis of phenolic substances, extracted by methanol, separated on C18 column using as mobile phase acetic acid (pH=3.1) in water-methanol and UV detection at 278 nm (Montedoro et al. 1992b);

– HRGC analysis of phenolic compounds, extracted by methanol, purified with hexane, derivatized with BSTFA (bis(trimethylsilyl) trifluoroacetamid) and determined on SE 30 capillary silica column, according to method described in a previous research (Angerosa et al., 1995).

3. RESULTS AND DISCUSSION

Table I details free acidity and peroxide value: data do not evidence any difference in quality of oil obtained by two kinds of decanters. Also the organoleptic test, performed by Panel tasters of Institute according to EC Regulation n. 2568/91, gave scores not significantly different, being the error of methode egual to +/- 0.5.

Table I

Sensory evaluation, chemical-physical characteristics and natural antioxidant contents of oils produced when processing olives in a dual-phase and a tri-phase decanter

VARIETY	Decanter kind (phase number)	Organoleptic evaluation (Panel test)	Acidity as % oleic acid	Peroxide value meq 0_2/Kg	Induction time (Rancimat method) h	Total tocopherols ppm	Total polyphenols as mg/l gallic ac.
Leccino	2	6.9	0.3	3	19.3	258.2	376
	3	7.2	0.3	3	14.3	248.9	242
Dritta	2	7.5	0.4	4	16.3	150.7	397
	3	7.3	0.4	4	13.3	105.4	270
Cipressino + Castiglionese	2	6.8	0.4	3	16.8	200.7	430
	3	6.9	0.4	4	14.5	180.4	359
More varieties	2	7.3	0.4	6	10.9	145.0	230
	3	7.3	0.4	7	7.9	132.8	113
More varieties	2	6.8	0.3	3	13.3	190.3	233
	3	7.1	0.3	4	8.7	171.7	115

On the contrary, considerable differences (Table I) were observed about the stability to oxidation-conventionally measured through the time that oil needs to become rank in fixed conditions of oxidation (Rancimat method). These findings involve the presence of antioxidant different levels in oils produced by two decanters under comparison.

Generally, just as the triglyceridic composition, tocopherols are related to oil resistance and it is very well known that their antioxidant activity increases from a to d (Slover et al., 1969). Nevertheless a difference of about 10% pratically in only a tocoferol content, always in favour of oils extracted by new decanter, does not justify the notable differences in induction time recorded for oils obtained by two kinds of decanter; in effect the very low (0.577) correlation coefficient shows, in an evident way, that the different stability of virgin olive oils has to be attributed to other antioxidant compounds. On the other hand, for a long time the literature reports (Gutierrez et al., 1965; Vazquez Roncero, 1978; Chimi et al., 1988) connect the oil resistance to autoxidation to total content of compounds which represent the polar fraction, conventionally known as polyphenols and quantitatively determined by conventional colorimetric method using the Folin-Ciocalteu reagent; the regression straight, calculated between contents of total polyphenols (Table I) and their corresponding induction times, shows a quite satisfactory correlation coefficient and high significance ($R=0.914$; $F=40.745$; $P<0.05$).

Levels of total polyphenols, as determined by the Folin-Ciocalteu method, evidence that the two extraction processing plants, which are different from one another only in the water quantity present in the system, significantly affect the phenolic fraction: in fact oils extracted by new decanter are, in all examined samples, 20-100% richer in polyphenols in comparison of oils produced by tri-phase decanter, because the water-oily layer interfacing is minimized (Table I). On the contrary, the use of conventional decanter needs to add a notable water quantitiy at about 30°C to dilute olive paste before its extraction; the water addition, considerably lowing the concentration of phenols in the aqueous phase, heavily modifies the partition equilibrium of the system with the result of a significant impoverishment of polyphenols in oily phase.

In the last decade high resolution liquid chromatography, in reverse phase and coupled with UV-Vis detection, was widely used to analyze phenols present in plants and in some foods (Mueller-Harvey et al., 1987; Ramírez-Martínez, 1988; Spanos and Wrolstad, 1990; Mc Rae et al., 1990). Particularly, the application of this technique in solvent gradient mode to virgin olive oils confirmed the polar fraction complexity (Solinas and Cichelli, 1982; Cortesi and Fedeli, 1983; Amiot et al., 1986; Tsimidou et al., 1992; Montedoro et al., 1992a; Akasbi et al., 1993), previously observed by other reseachers (Solinas, 1987), and allowed to perform the separation and quantitative determination of some phenol compounds, the total of which was related to oil stability (Montedoro et al., 1992b).

Therefore it is seemed useful to apply this technique to quantify phenols of oils produced by two kinds of decanter and to compare results obtained by HPLC analysis with those arising from Folin-Ciocalteu colorimetric method.

Figure 1 represents the chromatograms of polyphenols, as determined by HPLC, of oils extracted when processing olives in a dual-phase and a tri-phase decanter.

The two series of data (Table II) show the same trend and confirm the higher content in phenols of oils extracted by dual-phase decanter; also the increases observed are quite completely comparable with those obtained by the conventional analysis, and the regression straight, calculated between total levels of polyphenols determined by HPLC and their corresponding induction times, exibits an excellent correlation ($R=0.974$; $F=150.494$; $P<0.05$) which is similar to that obtained using for total phenols data concerning Folin-Ciocalteu determination.

It is proved by a study on the antioxidant activity of some simple phenols (Papadopoulos and Boskou, 1991) that tyrosol, even if present in rather high concentration in all virgin olive oil, has a very low antioxidant power and the compound that mainly contribute to oil stability is hydroxytyrosol, an o-diphenol compound.

Furthermore some researchers (Montedoro et al., 1992a) connected the oil resistence to autoxidation to only one compound, ascribable to an ester of elenolic acid and hydroxytyrosol, and attributed to this linked o-diphenol the not negligible role of the main antagonist of oxidation reactions. In addition to cited results, a very significant ($r=0.950$ and $F=73.502$; $P<0.005$) was found between induction times and the content of group of linked phenols having retention times ranging from 17.4 and 22.6, marked with M letter, that can be ascribed to esters of elenolic and both hydroxytyrosol and tyrosol, a unknown compound and an isomer of oleuropein aglycon (Table II) (Angerosa and Di Giacinto, 1995).

Therefore it is evident that, besides derivatives containing o-diphenols –the antioxidant activity of which is generally shared by all researchers–, the ester containing tyrosol plays a role especially active as antagonist of radicalic reactions of fatty oxidation; therefore it is obvious the need to separate and to quantify all compounds containing o-diphenols and also the tyrosol derivative for a right evaluation of virgin olive oil shelf-life.

Recently GC-MS (Angerosa et al., 1995) gave several useful information about polar compounds of oil. In fact it allowed to identify among simple phenols only tyrosol and hydroxytyrosol, even if pertinent

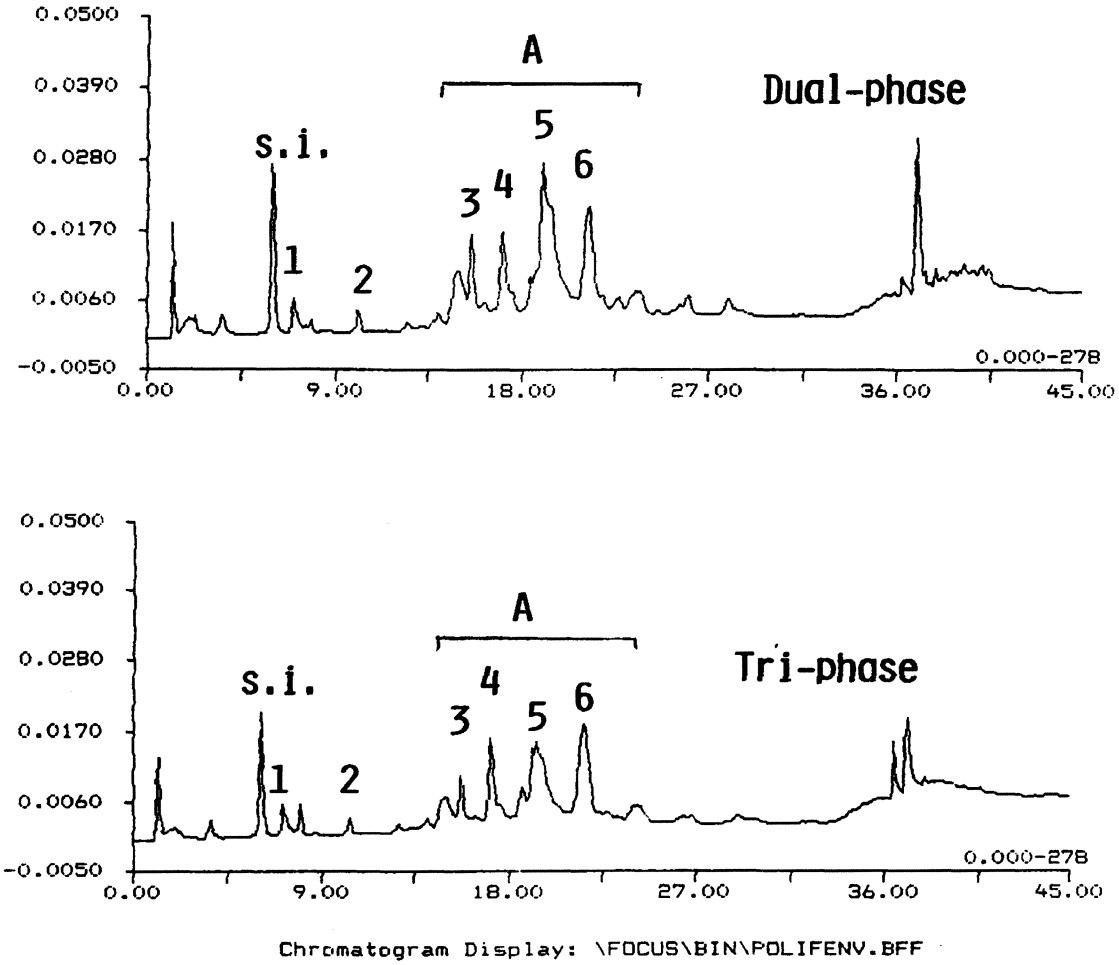

Figure 1
HPLC profile of phenolic compounds of virgin olive oils obtained from Dritta variety processed in a dual-phase and a tri-phase decanter. Peaks: s.i (internal standard) = gallic acid; 1) = hydroxytyrosol; 2) = tyrosol; 3) = ester of elenolic acid and hydroxytyrosol; 4) = ester of elenolic acid and tyrosol; 5) = unknown compound; 6) = oleuropein aglycon.

literature reports the presence of other simple phenols at very variable levels in the oils (Cortesi et al, 1981; Mc Rae et al., 1990); furthermore it allowed to assign to linked compounds extracted by methanol the definitive structure of phenolic derivatives and to establish wheter they are tyrosol or hydroxytyrosol derivatives. Finally, on the basis of mass spectroscopy data and results of mild acid catalyzed hydrolysis, GC-MS offered the possibility to define the chemical structure of many secoiridoid compounds ascribable to different structures, in equilibrium among them, of aglycons of glycosides that make up the natural store of fruits (Panizzi et al., 1960; Ragazzi et al., 1973; Kubo and Matsumoto, 1984; Gariboldi et al., 1986).

Knowledge about chemical structure of linked compounds that represent the most considerable part of total phenolic fraction, allows the right evaluation of level of o-diphenols through the addition of content of hydroxytyrosol and of linked compounds that it contains and that of aglycon of decarbomethoxy ligstroside, the above mentioned tyrosol derivative (Montedoro et al, 1993; Angerosa et al., 1995).

In order to have other information about the influence of decanter kind on the quali-quantitative composition of phenolic fraction, to resulting oils quantification by the gaschromatographic method was applied.

Figure 2 shows gas chromatograms of oils obtained when using a dual-phase and a tri-phase decanter.

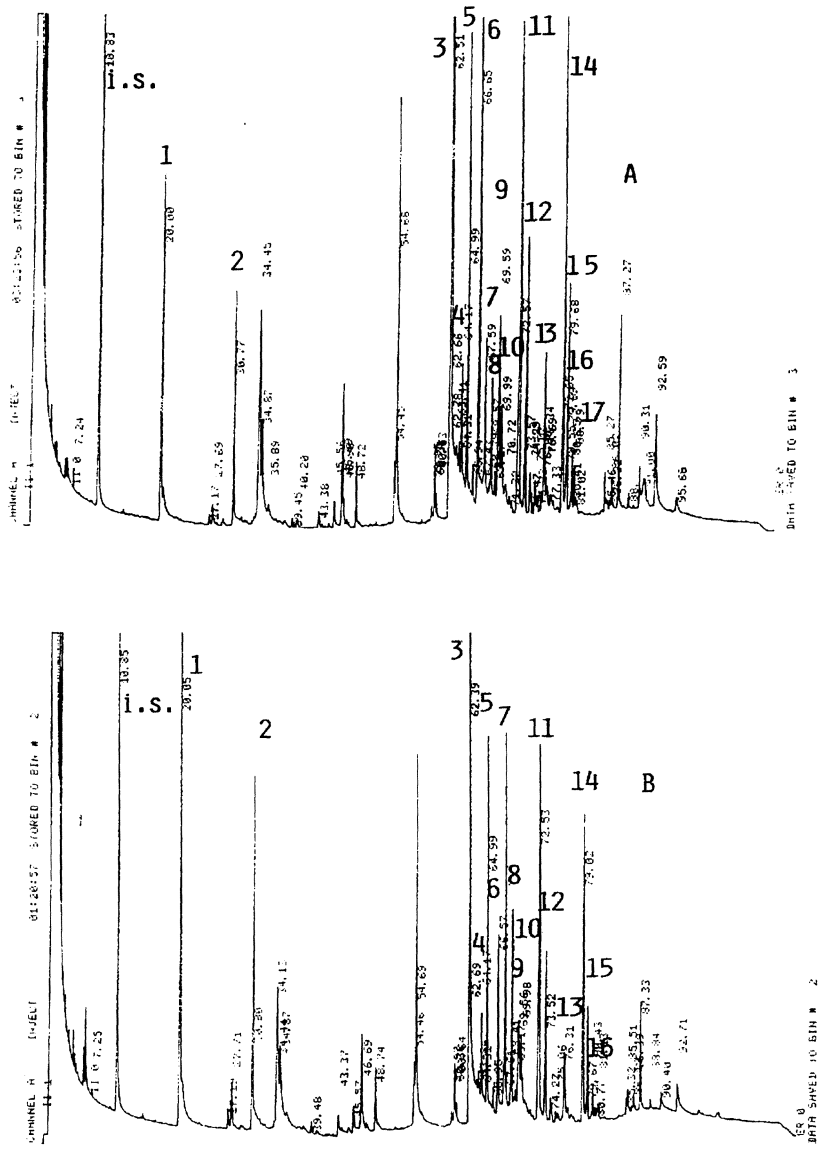

Figure 2
Gas chromatograms of phenolic compounds of virgin olive oils obtained from Dritta variety processed in a dual-phase (A) and a tri-phase decanter (B). Peaks: i.s. (internal standard) = resorcine; 1) = tyrosol; 2) = hydroxytyrosol; 3) = dialdehidic form of ligstroside aglycon containing no carbomethoxy group; 4), 5) and 9) = linked phenols containing tyrosol; 6), 8), and 10) = linked phenols containing hydroxytyrosol; 7) = monoglyceride; 11), 12) and 13) = linked phenols containing tyrosol; 14), 15), 16) and 17) = linked phenols containing hydroxytyrosol, ascribable to isomeric forms of oleuropein aglycon.

Trend is similar to those observed respectively for Folin-Ciocalteu and HPLC data (Table III); nevertheless the increases showed by oils extracted by dual-phase decanter are lower ranging between 10 and 50%, mainly because of different detection system. Furthermore it is to be added that gaschromatographic method allows to quantify all compounds having phenolic structure, with the sole approximation to make equal to 1 all reponse factors, while at this moment the HPLC method does not allow the right quantification, because some compounds were not characterized yet and in the conventional determination substances different from those phenolic could interfere because the reagent is not specific for phenolic functional group.

Table II

Induction times and contents of total polyphenols and some their components, determined by HPLC method, of oils obtained when processing olives in a dual-phase and a tri-phase decanter

VARIETY	Decanter kind (phase number)	Induction time (Rancimat method) h	Polyphenols as determined by Folin-Ciocalteu method*	Polyphenols as determined by HPLC method **	Hydroxytyrosol derivative **	A (phenolic compounds with Tr ranging from 17.4 and 22.6) **
Leccino	2	19.3	376	443.8	149.0	281.0
	3	14.3	242	279.8	31.4	117.3
Dritta	2	16.3	397	365.8	18.3	154.8
	3	13.3	270	230.0	4.3	132.7
Cipressino + Castiglionese	2	16.8	430	293.8	36.4	175.4
	3	14.5	359	263.8	29.2	155.1
More varieties	2	10.9	230	155.2	9.1	85.8
	3	7.9	113	88.0	0.4	49.2
More varieties	2	13.3	233	209.9	16.1	122.3
	3	8.7	115	95.6	0.8	24.9

* mg/l gallic acid
** ppm gallic acid

Table III

Induction times and contents of total phenolic compounds and some of them, determined by HRGC method as ppm resorcine, of oils extracted when processing olives in a dual-phase and a tri-phase decanter

VARIETY	Decanter kind (phase number)	Induction time (Rancimat method) h	Total phenolic compounds	o-diphenols	a (ligstrosid aglycon containing no carbomethoxy group)	0-diphenols + a
Leccino	2	19.3	310.8	151.2	21.7	172.9
	3	14.3	286.8	58.2	85.2	143.4
Dritta	2	16.3	197.2	114.5	2.8	117.3
	3	13.3	174.8	47.2	43.4	90.6
Cipressino + Castiglionese	2	16.8	306.1	111.0	49.9	160.9
	3	14.5	250.3	67.7	68.7	136.4
More varieties	2	10.9	145.5	45.1	40.5	85.6
	3	7.9	95.8	20.6	15.4	36.0
More varieties	2	13.3	216.8	23.1	109.6	132.7
	3	8.7	140.2	4.5	32.3	36.8

Data of Table III not only evidence that oils produced by dual-phase decanter have higher contents of total phenols but show, in all examined samples, higher concentrations of o-diphenols, both simple and linked, in comparison with oils obtained with tri-phase decanter: the wealth in o-diphenols is particularly considerable in the oils with a lower concentration of total phenolic compounds. This last result of extreme importance being the virgin olive oil shelf-life connected with this part of phenolic fraction and with aglycon of decarbomethoxy ligstroside, makes very profitable the oil production by a dual-phase decanter, especially from overripe fruits or cultivars characterized by polyphenol low levels.

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Recibido: Diciembre 1995
Aceptado: Febrero 1996