

BIOLOGICAL ACTIVITY OF SALIVA AGAINST *IN VITRO* LDL OXIDATION AFTER CHEWING COMMERCIAL CHEWING GUMS

ATTIVITÀ BIOLOGICA DELLA SALIVA CONTRO L'OSSIDAZIONE DELLA LDL
IN VITRO DOPO MASTICAZIONE DI GOMME DA MASTICARE COMMERCIALI

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ABSTRACT

The biological activity of the saliva from five different chewing gums, collected from six healthy volunteers, on the inhibition of low density lipoprotein (LDL) oxidation, produced *in vitro* by copper ions, was demonstrated and quantitatively expressed as % protection (% Pr). Vitamin E (20 μ M, 71.7% Pr), extract from the spearmint plant (50 mg, 63.0% Pr) and different concentrations of BHT synthetic antioxidant (0.014 μ g/mL, 9.7% Pr) were used for comparisons. Chewing quantities (0.6, 1.5 and 3 g) and length of chewing time (first 0.25, 1, 3 h, between

RIASSUNTO

È stata dimostrata l'attività biologica della saliva da cinque diversi tipi di gomme da masticare, prelevata da sei volontari sani, sull'inibizione dell'ossidazione della lipoproteina a bassa densità LDL, prodotta *in vitro* da ioni di rame che è stata espressa quantitativamente come la protezione % (% Pr). Sono state usate per confronto vitamina E (20 μ M, 71,7% Pr), estratta dalle piante di menta verde (50 mg, 63,0% Pr) e le varie concentrazioni dell'antiossidante di sintesi BHT (0,014 μ g/mL, 9,7% Pr). Sono state valutate delle quantità di gomme (0,6, 1,5 e 3 g) e lunghezza del tempo di ma-

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0.25th and 3rd h or between 6th and 7th h) were evaluated. Under the usual chewing conditions (1.5 g, 1 h) crude Chios mastic gum was found to be the most effective (74.6% Pr) followed by commercial Chios mastic gum (64.3% Pr) and spearmint-flavored gum (61.2% Pr), while the strawberry-flavored gum had a low effect (11.5% Pr). Biologically active substances present in Chios mastic gum (3 g) extracts and in the respective saliva (1 h chewing) were characterized as (poly)phenolic compounds in quantities of 0.3 and 0.2 mg, respectively.

sticazione (all'inizio 0,25, 1 e 3 ore, fra 0,25 fino a 3 ore e fra 6,0-7,0 ore). Sotto condizioni normali (1,5 g, 1 ora), è stata trovata che la pura mastica Chios è la più efficace (74,6% Pr) seguita poi dalla mastica commerciale Chios (64,3% Pr) e poi dalle cingomma aromatizzate al mentolo (61,2% Pr), mentre si è dato alla cingomma aromatizzata alla fragola un effetto basso (11,5% Pr). Le sostanze biologicamente attive presenti negli estratti nella gomma (mastica) Chios (3 g) e nella rispettiva saliva (1 ora di masticazione) sono state caratterizzate come composti (poli)fenolici in quantità di 0,3 e 0,2 mg, rispettivamente.

INTRODUCTION

There is increasing evidence that the association of blood total cholesterol with coronary heart disease appears to derive chiefly, if not entirely, from the low density lipoprotein (LDL) fraction with which it is highly correlated (GOLDSTEIN and BROWN, 1997). Furthermore, the oxidatively modified LDL stimulates the formation of foam cells, which lead to the formation of atherosclerotic plaques (STEINBRECHER *et al.*, 1990). Adequate dietary intake of antioxidants can help inhibit LDL oxidation and thus the development of atherosclerotic lesions.

The Mediterranean diet, rich in olive oil and in natural antioxidants (polyphenols, vitamins C and E, carotenoids) (BOSKOU, 1996a), has been shown to play a protective role against cardiovascular disease. For example, several polyphenols of the phenolic fraction of olive oil (VISIOLI and GALLI, 1994; VISIOLI *et al.*, 1998) and red wine (FUHRMAN *et al.*, 1995; KERRY and ABBEY, 1997) have been shown to inhibit the *in vitro*-induced LDL oxidation by metallic ions, while aqueous extracts of edible herbs from Crete, rich in polyphenols, have

been shown to protect cultured lung cells from *in vitro* oxidation (LIONIS *et al.*, 1998).

Crude Chios mastic gum is a natural resinous exudate obtained from the mastic tree (*Pistacia lentiscus* var. Chia cr. *Anacardiaceae*), which grows almost exclusively on the Island of Chios, Greece. It is obtained by making longitudinal incisions at close intervals from the base of the trunk up to the thicker branches. The resin has been used in traditional medicine for the treatment of various diseases such as gastralgia, dyspepsia and gastric ulcer (AL-SAID *et al.*, 1986), while its definite antibacterial activity against *Helicobacter pylori* has been well documented (HUWEZ *et al.*, 1998). In food processing it has been proven useful as a food preservative (WILLBLOCK, 1999) and antioxidant (ABDEL-RAHMAN and SOAD, 1975). Crude Chios mastic gum is supplied commercially either in the crude form as white or light yellow jelly crystals (1-10 mm diameter) or as sugar-coated or sugar-free chewing packs (1.5 g each). Other commercial chewing gums, also in 1.5 g packs, as sugar-free or sugar-coated forms, usually do not have a natural base; the majority of them

consist of synthetic polymeric bases, flavorings and other additives.

Millions of people chew commercial chewing gum every day, swallowing a considerable amount of saliva enriched with the extracts of the substances contained in the chewing materials. The possible positive or negative *in vitro* biological effect of this action against LDL oxidation was evaluated in the present study.

MATERIALS AND METHODS

Materials

All reagents were of analytical grade and solvents were of high performance liquid chromatography (HPLC) grade. Sterilized LDL lyophilised from 1 mL of LDL solution in 0.15 M NaCl and 0.01% EDTA at pH 7.4, with 6.3 mg protein per 400 mg LDL and phosphate buffer saline (PBS) tablets were purchased from Sigma (St. Louis, MO). Vitamin E (95%, prepared from synthetic phytol) and butylated hydroxytoluene (BHT) were also from Sigma. Malonaldehyde was from Aldrich (Steinheim, Germany). Thiobarbituric acid (TBA) was from Serva (Heidelberg, Germany). Folin Ciocalteu reagent was from Merck (Darmstadt, Germany). Plant spearmint was purchased from a local flea market in Kallithea (Athens, Greece). Chewing gums in commercial packs of approx. 1.5 g (sugar-free or sugar-coated) were bought from local stores in Kallithea, while the crude Chios mastic gum (jelly crystals) was kindly donated by the Mastic Gum Growers' Association (Chios, Greece). The spearmint-flavored chewing gum consisted of edulcorantes (mannitol, sorbitol, xylitol, aspartame and acesulfame K), gum base, E322 (lecithin), E422 (glycerine), E321 (BHT) and spearmint flavor. The strawberry-flavored chewing gum consisted of the same edulcorantes and gum base, plus E321, E322, E422, E330 (citric acid), E296 (malic

acid), E334 (tartaric acid), E472a (acetic acid esters of mono- and di-glycerides) and strawberry flavor.

Saliva collection

The Research Committee of the Harokopio University (Athens, Greece) approved the protocol for saliva collection. Saliva was collected at a dental clinic from six 25 to 65-year-old non-smoking, healthy volunteers with good mouth hygiene. Before being checked by the dentist, the participants had breakfast and brushed their teeth. Afterwards, they performed the chewing test, during which time eating, drinking or any other activities, except reading magazines, were not allowed. The tests took place on different days. All six volunteers participated in Tests No. 7 and 13 (Fig. 1), while five of them participated in Test No. 6 and only four of them in Tests No. 8-12, 14 and 15. All the tests were performed once for each volunteer. Several combinations of lengths of chewing time and chewing quantities were investigated. The saliva was not swallowed, but was collected in sterilized 20 mL glass tubes with glass cups, in volumes of approximately 15 ± 2 mL during the first 15 min (0.25 h) of chewing, 40 ± 5 mL during the first hour (1.0 h) of chewing or between the 6th and 7th hours of chewing, (6.0-7.0 h) and 60 ± 5 mL during the lengths of chewing time between 15 min and 3 hours (0.25-3.0 h) and 75 ± 5 mL during the entire period of chewing between zero-time and 3 hours (3 h) (the times given in parentheses are the denotations used hereafter). In accordance with a previous study (BOROS *et al.*, 1999) the gum chewing stimulation, which was common to all subjects, tended to minimize the differences in the rate of flow of saliva between the subjects. Tests No. 8 and 10 were performed on the same day and the saliva secreted during 0.25 h was collected separately from saliva se-

creted during 3.0 h. The same procedure described above was followed for Tests No. 11 and 12. For Test No. 2, which was defined as "blank saliva" test, secreted saliva was collected only during the last hour of a total seven-hour period of chewing. The saliva samples collected were immediately evaporated under a stream of nitrogen and evaporation was accomplished in a 37° C water bath, at a rate of approximately 5-6 mL per hour. The walls of the tubes were washed down with small volumes of ethanol and evaporated again. The residue was taken up in 0.01 mL ethanol and afterwards subjected to the LDL oxidation test.

LDL biological tests

LDL was kept at 2°C and its working suspension (200 µg protein/mL PBS) was prepared just before use. These precautions were taken since commercial LDL might be less stable than fresh LDL isolated from freshly drawn blood. The LDL oxidation test (LDL-ox) was performed as described by BALLA *et al.* (1991) with slight modifications. In brief, 1.0 mL of LDL working solution was mixed with 1.0 mL CuSO₄ (10 µM) as oxidizing agent, incubated at 37°C for 1, 3 and 6 h, and the resulting thiobarbituric acid reactant substances (TBA-RS), mainly malonaldehyde, were measured spectrophotometrically at 532 nm, after reaction with 2 mL of 1% alkaline TBA solution. Thiobarbituric Acid Reactive Substances (TBA-RS) were evaluated using a standard curve of standard malonaldehyde at different concentrations versus absorption at 532 nm. An LDL-blank test was also performed without the presence of copper ions. The LDL-ox and LDL-blank tests (Tests No. 1 and 16, respectively, Table 1) were carried out four times.

In Tests No. 2-15, the samples tested (saliva residues or standard samples of spearmint plant extract, vita-

min E and BHT) were added as 0.01 mL ethanol solutions with simultaneous addition of 0.99 mL CuSO₄, (instead of the addition of 1.0 mL only in the LDL-ox test described above). Saliva residues collected during Tests No. 2 (Table 1) and 6-15 (Fig. 1) were examined against LDL oxidation once, while Tests No. 3, 4 and 5 of reference samples were tested four times. The aforementioned 0.01 mL ethanol in the test mixture was found to exhibit no effect on the test results, which is in agreement with GORDON and WENG (1992).

Polyphenolic content

Total polyphenol content of saliva residue ethyl-acetate extract (3x5 mL) and of methanol/water (6:4, v/v) extracts (3 x 3 mL) from crude Chios mastic gum (0.5 g) and plant spearmint (0.5 g) were measured spectrophotometrically, as previously described (GUTFINGER, 1981). In brief, the above extracts were evaporated under a stream of nitrogen and the residues were diluted in 1 mL of ethyl-acetate or methanol, respectively. A portion of 0.1 mL of the 1 mL solutions reacted with the Folin Ciocalteu reagent and the products were measured spectrophotometrically at 725 nm. The results, expressed as ppm of caffeic acid, were calculated using a standard curve of caffeic acid in different concentrations (0-200 ppm) versus absorption at 725 nm. HPLC of the polyphenols from the saliva and crude Chios mastic gum extracts was performed according to a quaternary solvent gradient method, as previously described (ANDRIKOPOULOS *et al.*, 1989).

Statistical analysis

Statistical treatment of the results was performed by standard methods of statistical analysis (WALPOLE and MYERS, 1978), using a computer program for commuta-

Table 1 - Inhibitory effect against *in vitro* LDL oxidation, expressed as TBA-RS (μM), and % Protection (% Pr) of Chios mastic gum (CMG) saliva residues (SR).

Test No. (i)	Samples	Incubation period	Incubation period		
			1 h	3 h	6 h
1 (n=4) ^b	LDL-ox	TBA-RS ^a	1.412±0.292	2.294±0.352	2.694±0.345
		TBA-RS net ^{a,b}	1.412±0.292	2.193±0.437	2.471±0.387
2 (n=4) [*]	SR, blank-saliva [§]	TBA-RS ^a	1.396±0.206	2.279±0.479	2.672±0.256
		TBA-RS net ^{a,b}	1.396±0.206	2.179±0.544	2.449±0.309
		% Pr ^{a,c}	1.1±25.1	0.6±31.8	0.9±19.8
9 (n=4) [*]	SR after chewing (1.0 h) CMG (1.5 g), sugar coated	TBA-RS ^a	0.835±0.250	1.050±0.307	1.050±0.355
		TBA-RS net ^{a,b}	0.835±0.250	0.950±0.402	0.882±0.396
		% Pr ^{a,c}	40.8±27.2	56.7±27.0	64.3±22.4
15 (n=4) [*]	SR after chewing (1.0 h) crude CMG (1.5g)	TBA-RS ^a	0.700±0.288	0.740±0.027	0.850±0.358
		TBA-RS net ^{a,b}	0.700±0.288	0.640±0.260	0.627±0.399
		% Pr ^{a,c}	50.4±29.0	70.8±23.1	74.6±22.5
16 (n=4) ^c	LDL-blank	TBA-RS ^a	0.000±0.001	0.100±0.259	0.223±0.175

Significance tests (t-Tests)
 Test 1 versus Test 9 p < 0.00007 p < 0.000003 p < 0.0000008
 Test 1 versus Test 15 p < 0.00003 p < 0.00006 p < 0.0000003
 For % Pr, LDL-ox Test, LDL-blank Test and ^{a,b,c} superscripts see "Materials and Methods" section.
 TBA-RS = thiobarbituric acid reactant substances.
[†] n=number of replications.
^{*} n=number of subjects.
[§] Saliva collected during 6.0-7.0^h of a total seven-hour continuous chewing of 3.0 g sugar coated CMG.

tions (ANOVA Toolkit application of MS Excel). The results in Table 1 are presented as 99% confidence intervals, calculated using the following formulae (in the order of ^{a,b,c} superscripts in Table 1):

$${}^a \text{TBA-RS}_i \pm [99\% \text{ Confidence Limits}]_i$$

$$[99\% \text{ Confidence Limits}]_i = t_{v,a} \times \text{SD}_i / n^{1/2}$$

$${}^b \text{TBA-RS}_{\text{net } i} = \text{TBA-RS}_i - \text{TBA-RS}_{16}$$

$$\text{SD}_{\text{net } i} = (\text{SD}_i^2 + \text{SD}_{16}^2)^{1/2}$$

$$\text{and } [99\% \text{ Confidence Limits}]_{\text{net } i} =$$

$$= t_{v,a} \times \text{SD}_{\text{net } i} / n^{1/2}$$

$${}^c \% \text{Pr}_i = (\% \text{ Protection of test } i) =$$

$$= (\text{TBA-RS}_{\text{net } i} - \text{TBA-RS}_{16}) \times 100 / \text{TBA-RS}_{\text{net } i}$$

$$\% \text{SD}_i = (\text{SD}_{\text{net } i}^2 + \text{SD}_{\text{net } 16}^2)^{1/2} \times 100 /$$

$$/ \text{TBA-RS}_{\text{net } i}$$

$$\text{and } [99\% \text{ Confidence Limits}]_{\% \text{Pr } i} =$$

$$= t_{v,a} \times \% \text{SD}_i / n^{1/2}$$

where: the subscript *i* is the serial number of the test (see first column of Table 1); TBA-RS_{*i*} = mean value of TBA-RS obtained in Test No. *i*; SD_{*i*} = standard deviation of all the experimental TBA-RS values of Test No. *i* (so SD_{*i*} / n^{1/2} equals the

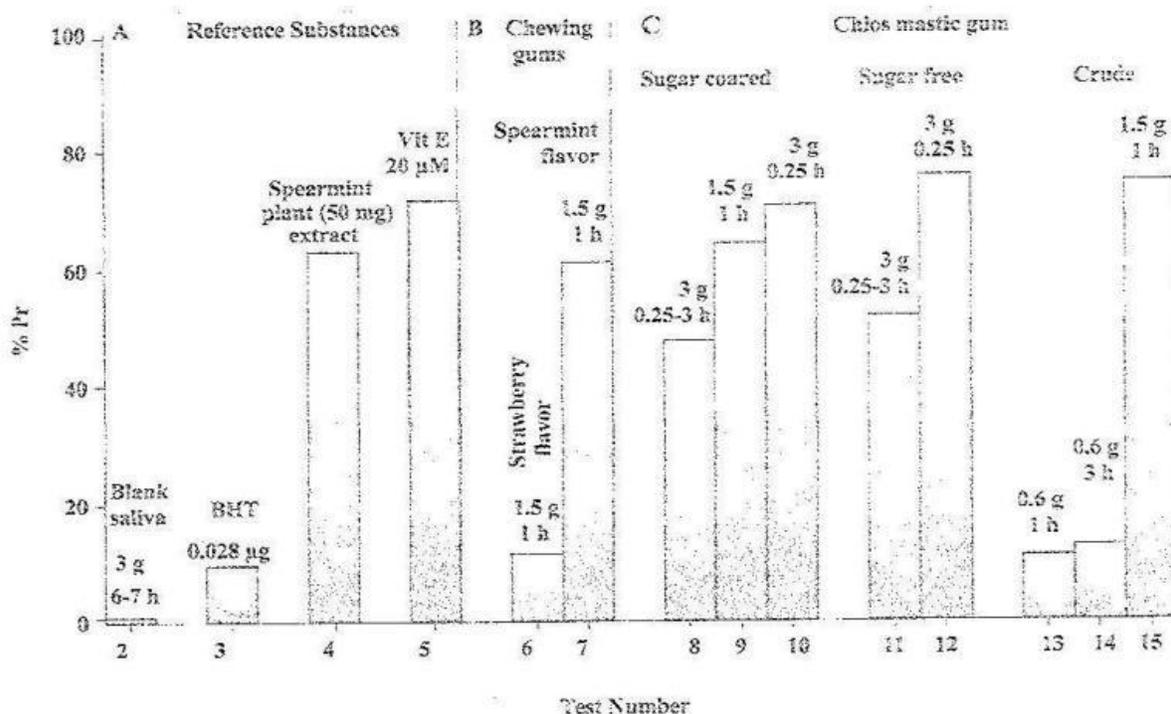


Fig. 1. - Protective (%) effect of (A) reference substances, (B) chewing gum saliva residues and (C) Chios mastic gum saliva residues, against the *in vitro* LDL oxidation.

% Pr: % Protection based on results of 6 h-incubation (see Table 1 and "Statistical analysis" subsection). The quantities (g) of gums used for chewing and the respective lengths of chewing time (h) are given above the bars. BHT: Butyl-hydroxy-toluene, 0.028 µg in the test cuvette [equivalent to 200 ppm in chewing gum transferred in blood circulation]. Vit E: Vitamin E, 20 µM in the test cuvette. Tests No. 1 (LDL-ox) and No. 16 (LDL-blank) exhibited the lowest and highest % Pr, respectively, and were used as the lower and upper limits for the evaluation of the other Tests (No. 2-15).

The 99% confidence limits (computed as described in the subsection "Statistical Analysis") are as follows: Test No. 2 = ±19.8; Test No. 3 = ±20.1; Test No. 4 = ±19.6; Test No. 5 = ±19.6; Test No. 6 = ±20.1; Test No. 7 = ±20.9; Test No. 8 = ±20.7; Test No. 9 = ±22.4; Test No. 10 = ±20.1; Test No. 11 = ±19.5; Test No. 12 = ±20.1; Test No. 13 = ±26.3; Test No. 14 = ±35.0; Test No. 15 = ±22.5.

standard deviation of the mean value $TBA-RS_i$; $t_{v,a}$ = critical t-distribution factor for $v = (n - 1)$ degrees of freedom and 100 $(1 - 2\alpha)$ % confidence (e.g. for 99% confidence limits applied throughout the present report, $v = (n - 1) = 3$ and $t_{v,a} = 5.841$); $TBA-RS_{net i}$ and $SD_{net i}$ = respective values of the net of Test No. i oxidation derived by subtracting the mean value of TBA-RS of the LDL-blank from the respective mean value of Test No. i ; and, $\%Pr_i$ and $\%SD_i$ = respective "relative" values of the percent protection of Test No. i . $\%Pr_i$ is an indicative value expressing the relative inhibitory action of saliva, collected after chewing gum i , on the

LDL oxidation produced *in vitro* by copper ions (Test No. 1).

Thus, e.g. for Test No. 9 (6 h incubation):

Average $TBA-RS_i = 1.050$, $SD_i = 0.1215$ and 99% Confidence Limits for $TBA-RS_i = (5.841 \times 0.1215) / 2 = 0.355$. Net oxidation $TBA-RS_{net 9} = (1.050 - 0.223) = 0.822$, $SD_{net 9} = (0.1215^2 + 0.0599^2)^{1/2} = 0.1350$, and 99% Confidence Limits for $TBA-RS_{net 9} = (0.1350 \times 5.841) / 2 = 0.396$. $\%Pr_9 = (2.471 - 0.822) \times 100 / 2.471 = 64.3$, $\%SD_9 = (0.1350^2 + 0.1326^2)^{1/2} \times 100 / 2.471 = 7.658$, and 99% Confidence Limits for $\%Pr_9 = (7.658 \times 5.841) / 2 = 22.4$.

RESULTS AND DISCUSSION

The biological tests for two representative saliva residue samples after chewing different Chios mastic gums under the usual chewing conditions (1.5 g, 1.0 h), are presented in Fig. 2, together with the LDL-ox test, the "blank-saliva" test and LDL-blank test. The protecting effect of the saliva residue from Chios mastic gum is evident. The "blank-saliva" results are reported, because, after six hours of continuous chewing, all the biologically active compounds from the mastic gum are expected to be swallowed. This was confirmed by the absence of any protective effect on LDL protection from the respective saliva secreted between the 6th and the 7th hour of continuous chewing. This is also indicative of the upper limit of length of chewing time, beyond which the protective effect is negligible. The choice for the

collection of the saliva secreted during chewing as "blank saliva", instead of the resting saliva, was made, because the masticatory movements provide stimuli to the human parotid gland, which enhances saliva secretion (KERR, 1960). More importantly, mastication produces almost the same amount of secretion among the subjects under a certain stimulation (BOROS *et al.*, 1999). In Table 1 the percentage of protection (% Pr) calculated for some indicative samples of saliva residues from Chios mastic gum are also presented. After six-hours of incubation, TBA-RS values tended to stabilize (data not shown), thus no further incubation times were used. Therefore, the % Pr refers to 6 h incubation time.

The protective effect against the *in vitro* LDL oxidation of the saliva residues from six volunteers after chewing five different chewing gums, in different quantities and for different lengths of

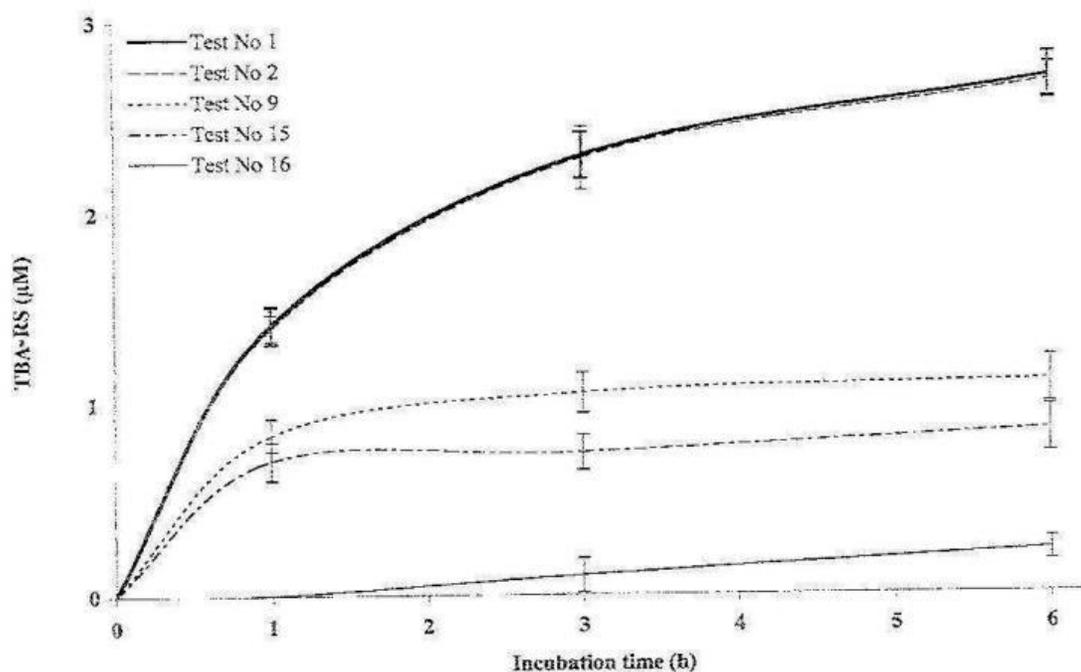


Fig. 2 - Biological activity (TBA-RS) against copper sulfate induced LDL oxidation of representative samples of Table 1: Test No. 1 = LDL-ox; Test No. 2 = SR "blank-saliva"; Test No. 9 = SR from CMG, sugar coated; Test No. 15 = SR from crude CMG; Test No. 16 = LDL-blank. Abbreviations and other conditions as in Table 1.

time, is reported in Fig. 1. Equal chewing amounts and lengths of chewing time of the chewing gums examined (1.5 g, 1.0 h) showed that the crude Chios mastic gum (Test No. 15) gave the highest protective activity on LDL oxidation, followed by the sugar-coated Chios mastic gum (Test No. 9), the commercial spearmint-flavored gum (Test No. 7) and the commercial strawberry-flavored gum (Test No. 6). The inhibitory effect of the spearmint-flavored gum is probably due to the spearmint, as shown by the percentage protection of Tests No. 7 and 4. Since the BHT content was not reported on the spearmint-flavored gum label, the additive was tested against LDL oxidation in the different final concentrations 0.014, 0.035, 0.07, 0.14, 0.28 and 0.56 $\mu\text{g}/\text{mL}$. These concentrations correspond to a possible migration of BHT in human blood (4.5 L) circulation (200 mg LDL / dL blood) from 200, 500, 1,000, 2,000, 4,000 and 8,000 ppm BHT in gum, respectively. These BHT concentrations exhibited dose dependent % Pr values as follows: 9.7 ± 20.1 , 14.3 ± 21.6 , 22.7 ± 20.1 , 28.7 ± 19.5 , 47.9 ± 19.5 and 55.6 ± 19.8 , respectively. The 200 ppm concentration was the most appropriate for comparison, since it represents the usual upper limit in foods. It seems that the spearmint flavor contributed the most to the inhibitory effect, unless the BHT in the gum was close to the unusual 8000 ppm concentration. Whether the E422 (glycerin) additive contributed to the antioxidant activity requires further investigation. The E322 (lecithin) additive, which is also contained in the strawberry-flavored gum, appeared to have a negligible effect, along with the strawberry-flavored gum (Test No. 6) which had only 11.5% Pr, the lowest among the tested gum saliva residues.

Comparing the different chewing quantities (Tests No. 13 and 15) on the % Pr effect under similar conditions in-

dicated that the protective effect depends on the chewing quantity. Thus, an increase in the chewing quantity of crude Chios mastic gum (jelly crystals) from 0.6 g to 1.5 g resulted in an approx. 54% higher protective effect. For comparable quantities and types of Chios mastic gum (2 packs = 3 g, sugar-coated), by comparing the effects during the first 0.25 h of chewing (Test No. 10) with those in the period between 0.25 and 3.0 h of continuous chewing (Test No. 8), 59.7% of the protective compounds were eluted from the chewing material during the first 15 min of chewing. These results are almost identical to the results obtained in Tests No. 11 and 12 (59.3%), using sugar-free gum under respective chewing conditions. By also taking into account the results of Tests No. 13 and 14, it appears that about 85% of the protective substances are eluted from the chewing material during the first hour of chewing.

Comparing Tests No. 8 and 11 (0.25-3.0 h chewing) and Tests No. 10 and 12 (0.25 h chewing), it can be concluded that after chewing 2 packs of commercial Chios mastic gum, the sugar-free gum gave approx. 5% higher protection against LDL oxidation than the sugar-coated gum. Since the sugar-free Chios mastic gum also has a coating of natural sweeteners, it still has to be investigated if the slight variation is due to the sugar or the sweeteners. Additionally, in comparing Tests No. 9 and 15 the commercial and crude Chios mastic gum gave similar % Pr. The slight difference (10.3%) is probably due to the slight differences in weight (approx. 1.5 g) or the additives in the sugar-coated pack.

The inhibitory effect of Chios mastic gum (Test No. 15) under the usual conditions of chewing was found to be almost equal to that of the optimum dose of vitamin E (Test No. 5) (GORDON and WENG 1992). After six hours of chewing (and probably much earlier), all the bio-

logically active substances in Chios mastic gum had disappeared (Test No. 2) also indicating that pure saliva has no protective effect on LDL oxidation.

The active substances in Chios mastic gum and in saliva after chewing Chios mastic gum were isolated by solvent extraction. In these extracts the presence of phenolic compounds was determined colorimetrically, while the similarity of the individual phenols in both extracts was proven by the same peak sequence and retention times after HPLC analysis (data not shown). HPLC peak identification is now in progress, combined with the respective bioavailabilities, and the results will be published in the future. Both extracts also exhibited biological activity in LDL oxidation tests, comparable to Tests No. 9 and 15 in Fig. 2.

Total polyphenols (0.3 mg) from Chios mastic gum (3 g, 2 packs) were comparable to the amount (0.2 mg) found in the saliva from chewing the same amount of Chios mastic gum (3 g, 1 h chewing). The latter corresponds to 0.1 mg polyphenols from 1.5 g, 1 h chewing (64.3% Pr, Test 9). This is almost equal to the amount of polyphenols estimated (0.066 mg) in the 50 mg of spearmint plant extract (63.0% Pr, Test 4). This indicates a possible relationship between the percentage protection and the polyphenol content in both Chios mastic gum and the spearmint plant extract.

Recently, a distinct effect of several polyphenol-rich extracts of Chios mastic gum against *in vitro* LDL oxidation has been documented (ANDRIKOPOULOS *et al.*, 2002). Compared to olive oil, which is the main source of fat in Mediterranean countries (20-40 g olive oil/day/person) and which is known to be rich in polyphenols (50-200 mg/kg) (BOSKOU, 1996a), the phenolic contribution of Chios mastic gum to the daily intake of phenols is indeed valuable. It amounts to approximately 10% of that from olive oil, considering that the amount from olive oil is 1 (VISIOLI *et al.*, 1998) to 2

mg/day/person (BOSKOU, 1996b). In addition, chewing two packs of Chios mastic gum amounts to 1-5% of the overall daily intake of polyphenols, when considering an intake of 23 mg flavonols/day/person (HERTOG *et al.*, 1993), but quite a bit lower when considering 1 g/day/person (SCALBERT and WILLIAMSON, 2000). In Mediterranean countries, the dietary antioxidants, when consumed over long time periods, have been suggested as being able to reduce mortality due to cardiovascular heart disease (HERTOG *et al.*, 1993).

These findings support the relationship of Chios mastic gum to the beneficial effects of the polyphenol-containing foods and beverages e.g. olive oil, wine, herbs and species and other vegetables most of them present in the Mediterranean diet. For example, the protection of LDL by olive oil polyphenols has been well documented by *in vitro* experiments (VISIOLI and GALLI, 1994) and by *ex-vivo* experiments on rabbits (WISEMAN *et al.*, 1996) and humans (AVIRAM and ELIAS, 1993). Other polyphenolic compounds isolated from black or green tea extracts (YANG *et al.*, 2000) and from human saliva after consumption of tea (YANG *et al.*, 1999a) have also been reported with biological activities, *in vitro* e.g. in cancer prevention. Bioavailability of olive oil polyphenols has been partially estimated for tyrosol (MIRO CASAS *et al.*, 2001; VISIOLI *et al.*, 2000) and hydroxytyrosol (VISIOLI *et al.*, 2000) and for tea polyphenols for catechins (YANG *et al.*, 1999b). The aforementioned biological properties, combined with respective bioavailabilities, whenever demonstrated unequivocally *in vivo*, may partially explain the beneficial effect of phenolic and polyphenolic compounds, as already pointed out by SCALBERT and WILLIAMSON (2000). Until now, estimation of some certain biological activities *in vitro* remains the only evidence for a possible link between the phenolic content of Chios mastic gum and health benefits, while *in vivo* and *ex*

in vivo investigations are an area with promising prospects.

Chios mastic gum is a local Mediterranean product and, apart from its consumption as chewing gum, also has several other uses, especially in Greek, Turkish and Arabic cooking, e.g., in powder form as a food additive, in the form of a sugar containing gel as a table-sweetener and in the form of mastic oil as a beverage and sweet additive. It would be expected that these uses of Chios mastic gum would give greater protective activity than chewing gum, as in these cases the gum is directly swallowed. Nevertheless, saliva from chewing could also be considered to make a marked contribution to the average intake of Chios mastic gum products, because for many people chewing is a daily habit in contrast to the other products, which are consumed only occasionally. The polyphenolic intake from chewing Chios mastic gum also has the advantage of the "net intake" e.g. without the triglyceride content of olive oil, which contributes to the daily intake of lipids.

CONCLUSIONS

In conclusion, saliva secreted during chewing of crude Chios mastic gum was proven to be the most effective in protecting LDL from *in vitro* oxidation, compared to other commercial chewing gums with synthetic polymeric base and flavor/antioxidant additives. To obtain the greatest possible health benefits, the length of chewing time should be over 15 min, though not more than 1 h.

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