PAPER

PROTECTION OF TRADITIONAL GREEK FOODS USING A PLANT EXTRACT

SALVAGUARDIA DEGLI ALIMENTI TRADIZIONALI GRECI UTILIZZANDO UN ESTRATTO VEGETALE

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ABSTRACT

The effect of rosemary extract on two traditional Greek foods (Tarama salad and Deples) was evaluated using the Rancimat method and sensory evaluation. The rosemary extract at 400 ppm gave the highest protection against lipid oxidation of Tarama salad during 40 days of storage. It was superior to BHA (200 ppm). The extract at a concentration of 150 ppm protected Deples from oxidation during 35 days of storage. Panelists clearly preferred products with the rosemary extract.

RIASSUNTO

È stato valutato l'effetto di un estratto di rosmarino su due tradizionali alimenti greci (l'insalata Tarama e il Deples) utilizzando il metodo Rancimat e la valutazione sensoriale. L'estratto di rosmarino a 400 ppm esplicava la migliore protezione contro l'ossidazione lipidica dell'insalata Tarama durante 40 giorni di conservazione e risultava superiore al BHA (200 ppm). L'estratto ad una concentrazione di 150 ppm proteggeva il Deples dall'ossidazione durante 35 giorni conservazione. Gli assag-

- Key words: deples, induction period, rosemary extract, sensory evaluation, Tarama salad -

The shelf-life of products was calculated using the linear adjustment equation. Foods with rosemary had 17-102% longer shelf-life.

giatori preferivano chiaramente prodotti contenenti l'estratto di rosmarino. La shelf life dei prodotti veniva calcolata utilizzando un'equazione lineare. Gli alimenti con estratto di rosmarino presentavano una shelf life più lunga del 17-102%.

INTRODUCTION

Traditional foods contribute significantly to the human diet in many countries around the world since they are closely related to the local culture. Some of them are produced by simply combining the ingredients and then mixing them (e.g. Tzatziki salad), while others, after mixing, undergo further processing such as frying (e.g. pan cakes) or baking (e.g. baklava). Tarama salad and deples are traditional Greek foods. Tarama salad is a traditional delicacy that looks like a pink or red paste; it is consumed mainly during the forty-day period of fasting before Easter. It is made of oil, fish roe, bread crumbs or bread and water, and is stored at 6°-8°C. It is a traditional home-made food, but it can also be purchased in supermarkets. The lipid phase accounts for more than 50% of the salad mass. Within two weeks, oxidation makes the product unacceptable, especially when the salad is stored unsealed in the fridge.

Deples is another traditional Greek delicacy (which comes in the form of a swirled or twisted crispy pastry); it is mainly consumed during the Christmas holidays. It is made of flour, orange juice and whole egg to produce a dough which is then fried in vegetable oil and stored at room temperature for up to two weeks. It contains a high amount of absorbed oil, which oxidises easily making it commercially unacceptable. The use of highly unsaturated vegetable oil (e.g. soybean oil) increases the susceptibility to oxidation (RUDNIK *et al.*, 2001).

Lipid peroxidation, which involves a series of free radical-mediated chain reaction processes, is also associated with several types of biological damage. The role that free radicals and active oxygen plays in causing atherogenesis in humans is becoming clearer (KISHK and EL-SAYED, 2007). Antioxidants are used to reduce or retard deterioration of the lipid phase. In addition, the use of antioxidants minimises discoloration, and off-flavours and contributes to emulsion stability which can be negatively affected by the degradation of lipids in foods (MITSUMOTO *et al.*, 2005; KISHK and EL-SAYED, 2007). ESTEVEZ *et al.* (2007) reported that artificial antioxidants (usually butylated hydroxyanisole-BHA) are added to food to inhibit lipid oxidation and obtain a longer shelf-life. Synthetic antioxidants are not popular among consumers, since it has been shown that they act in promoting carcinogenesis and mutagenesis (FARAG *et al.*, 1989). On the other hand, plant extracts especially rosemary (*Rosemarinus officinallis* L.) and sage (*Salvia officinallis* L.) provide varying levels of antioxidant activity, depending on the method of assessment in different fats and oils (CHE MAN and TAN, 1999; KIM *et al.*, 1994; ZHANG *et al.*, 1990). In an earlier study the effect of the natural extract from rosemary, dissolved in frying oil, was evaluated against the oxidation of oil, during intermittent frying of potato chips (LALAS and DOURTOGLOU, 2003). In the present work, rosemary extract was used to protect the lipid phase of the two tradition-

al Greek foods mentioned, prepared under different temperature conditions and storage. This time, the antioxidant was added to the food ingredients and not to the frying oil.

The main objectives of this study were: first, to assess the oxidative susceptibility of two traditional foods (Tarama salad and Deples) prepared under different conditions (high and low temperature) and second to evaluate the possibility of slowing this oxidation by addition of a natural extract obtained from rosemary. Extension of the shelf-life was also predicted. The potential substitution of the synthetic antioxidant BHA (normally used in commercial preparations) in Tarama salad was also examined.

MATERIALS AND METHODS

Materials

Rosemary extract (Rosemax®) is a commercial product (Vioryl Chemical and Agricultural Industry Research S.A., Afidnes, Greece) with the following composition: 43% caffeic acid, 7% ferulic acid, 10% carnosic acid, 20% rosmarinic acid, 5% vanillic acid, 5% carnosol, 3% p-hydroxy benzoic acid and 7% ursolic acid (LALAS and DOURTOGLOU, 2003). Butylated hydroxyanisole (BHA) was obtained from Sigma Chemicals Company Co. (St. Louis, USA). Soybean oil (Kope, Athens, Greece), bread flour (Jotis S.A., Athens, Greece), fresh oranges and eggs were purchased from the local market. Salted roe of *Gadidae* spp. was provided by Olympus Foods S.A. (Nea Efessos, Pieria, Greece).

Tarama salad recipe

The salad recipe used was: 65% soybean oil, 8% salted roe, 26% water, 1% breadcrumbs and a selected amount of antioxidant (extract or BHA). The ingredients were mixed using a Vorwerk Thermomix 3300 (Vorwerk France s.a., Paris). The salad was poured into plastic containers (200 g per container) and stored unsealed at 5°C for 40 days. In total, 10.0 kilograms of salad were divided into five different batches (produced by adding: 0, 100, 200, 400 ppm rosemary extract, and 200 ppm BHA, respectively) and stored at 6°C in the dark. Two containers of each batch were removed from cold storage each day. The first container was subjected to sensory evaluation (day 0 and 40). The lipid phase from the salad of the other container was extracted with n-hexane (to determine the oxidative condition) and centrifuged for 10 min at 6,500 rpm using a Sorvall General-Purpose RC-3 Automatic Refrigerated Centrifuge (Ivan Sorvall Inc., Newtown, CT, USA). Its oxidative condition was then determined using the Rancimat method. Induction period was evaluated at day 0, 10, 20, 30 and 40.

In total three replicate experiments were carried out (using the same recipe and experimental conditions for all determinations).

Deples recipe

Deples were prepared using the following recipe: 63% bread flour, 17% orange juice and 20% whole egg (about 9 eggs). The ingredients were mixed with mixer (Vorwerk Thermomix 3300) and the dough was divided into four batches. Rosemary extract at concentration levels of 0, 50, 100 and 150 ppm were added. Each batch was then passed through a pasta machine (Roma/Weston Pasta Machine 6in - Model 150P, Pragotrade USA, Inc. Strongsville, Ohio) and cut into parallelogram pieces of uniform thickness (15x5 cm). Each piece was then properly swirled to form a helical shaped piece before frying.

In total three replicate experiments were carried (using the same recipe and experimental conditions for all determinations).

Frying method of Deples

Frying was performed in four different 22 cm diameter pans using soybean oil. Temperature was monitored with an IKA Labortechnik ETS-D4 fuzzy (IKA Labortechnik, Germany) digital thermometer. Each batch of Deples was deep-fried independently in 1 L of soybean oil. The batches of Deples were put in the oil when the temperature reached 170°C and fried for 2 min. After frying they were placed on absorbent paper to remove the excess oil. Sample batches were packed in unsealed plastic bags and kept in the dark at 4°C for 35 days.

Deples were subjected to sensory evaluation (day 0 and 35) and determination of the oxidative condition of the lipid phase was determined over a six-week period (day 0, 7, 14, 21, 28 and 35). The lipid phase was extracted from ground Deples (using a Krups GVA2 Mini-chopper, Zentralkundendienst Krups GmbH, Solingen, Germany) with 250 mL of n-hexane in conical flasks for 30 min, in the dark. The oil was collected after the solvent was evaporated, under vacuum using a rotary evaporator (Büchi Rotavapor R-215 and B-160 Vacobox pump-BÜCHI Labortechnik AG, Flawil, Switzerland). The efficiency of oil extraction was 98-100%. Induction period was measured after extraction.

Determination of the oxidative state of foods

The determination of the induction period was determined by the Rancimat method (100°C and 20 L/h) adapted from LALAS and DOURTOGLOU (2003).

Sensory evaluation of products

Deples and Tarama salad were placed in plastic dishes to evaluate their sensory quality using the method described by TSAKNIS and LALAS (2002). Twenty panellists were selected from laboratory staff. Each sample was coded with a three-digit code number. Panellists evaluated the overall acceptability of each sample (taking into account any flavour of rancidity and undesirable taste) using a numerical scale of 1 to 9 (with 1 = not acceptable, 9 = extremely good) as well as the colour by using a numerical scale of 1 to 4 (with 4 = white-yellow, 1 = brown for Deples and 4 = red, 1 = light pink for Tarama salad). In addition, panellists had to state their overall preference taking into account any flavour of rancidity and unpleasant taste or colour.

Statistical analysis

The results are the mean values of triplicate analyses of three different experiments (triplicate analysis per experiment). The statistical significance of the differences between the mean values was assessed by ANOVA. Changes in the induction period of the lipid phase of Tarama salad and Deples were correlated with the time past since the production date by a linear regression equation ($Y = a + bX$), where Y = induction period and X = number of days since the production date. By applying the developed linear equations in which shelf-life corresponded to a value for an induction period of 10 hours (Tarama Salad) or 1 hour (Deples), the effective shelf-life of each product was expressed in days before the product became unacceptable.

RESULTS AND CONCLUSIONS

Tarama salad

Oxidation of the lipid phase of the Tarama salad was inhibited by both rosemary extract and BHA. The results showed that, the longer the salads were kept in storage, the shorter was the induction period (Table 1). The reduction of induction period for all batches was significant ($P<0.05$) in all cases. The rose-

Table 1 - Changes in induction period* of the lipid phase Tarama salad samples (0, 100, 200, 400 ppm of rosemary extract and 200 ppm BHA) during 40 days of storage.

Day	No antioxidant	Extract 100 ppm	Extract 200 ppm	Extract 400 ppm	BHA 200 ppm
0	$37.4\ (1.2)^{a}_{a}$	$38.0\ (1.7)^{b}_{a}$	$41.9\ (1.7)^{c}_{a}$	$42.0\ (1.2)^{c}_{a}$	$39.0\ (1.2)^{d}_{a}$
10	$33.2\ (1.5)^{a}_{b}$	$34.6\ (1.2)^{b}_{b}$	$37.0\ (1.0)^{c}_{b}$	$37.9\ (1.3)^{c}_{b}$	$35.6\ (1.4)^{b}_{b}$
20	$30.5\ (1.2)^{a}_{c}$	$33.3\ (1.0)^{b}_{c}$	$35.1\ (1.3)^{c}_{c}$	$36.7\ (0.6)^{d}_{c}$	$34.0\ (1.6)^{b}_{c}$
30	$25.3\ (1.2)^{a}_{d}$	$31.0\ (1.9)^{b}_{d}$	$33.8\ (1.0)^{c}_{d}$	$35.4\ (0.7)^{d}_{d}$	$31.9\ (1.5)^{b}_{d}$
40	$17.5\ (1.3)^{a}_{e}$	$25.2\ (1.0)^{b}_{e}$	$31.9\ (0.9)^{c}_{e}$	$33.0\ (1.3)^{d}_{e}$	$26.4\ (0.7)^{b}_{e}$

* Values are the mean and standard deviation (in parentheses) of triplicate determinations of three replicate experiments carried out simultaneously (nine determinations in total). Means within each row with different superscripts are significantly (P<0.05) different. Means within each column with different subscripts are significantly (P<0.05) different.

mary extract at a concentration of 400 ppm was the most effective antioxidant followed by the extract at 200 ppm after 40 days of storage. BHA (200 ppm) showed antioxidant activity equal to the extract at 100 ppm (no significant difference at P<0.05).

These results were also reflected in the sensory evaluation of Tarama salad after 40 days of storage (Table 2). On the day of preparation (day 0), the overall acceptability was good for all samples (no significant difference at P<0.05). After 40 days of storage the batches containing 200 and 400 ppm of extract had the highest (significant at P<0.05) overall acceptability among all batches and no significant change (P<0.05) in colour. The other batches showed a significant (P<0.05) reduction in their overall acceptability. In addition, no discolouration was observed in the Tarama salad with rosemary extract at 200 or 400 ppm. At day 40, the colour of the batches of salad with rosemary extract at 100 ppm, BHA or with no antioxidant showed significant (P<0.05) changes from red to pink. As determined by the overall preference, on the last day of the experiment the panellists showed a clear pref-

Table 2 - Sensory scores of Tarama salad after 40 day of storage.

Sensory attributes	Day	No antioxidant	Extract 100 ppm	Extract 200 ppm	Extract 400 ppm	BHA 200 ppm	
Overall acceptability*	0	$9\ (0.0)^{a}_{a}$	$9\ (0.0)^{a}_{a}$	$9\ (0.0)^{a}_{a}$	$9\ (0.0)^{a}_{a}$	$9\ (0.0)^{a}_{a}$	
	40	$3\ (1.0)^{a}_{b}$	$5\ (1.0)^{a}_{b}$	$8\ (1.0)^{b}_{b}$	$8\ (0.9)^{b}_{b}$	$7\ (0.9)^{c}_{b}$	
Colour**	0	$4\ (0.0)^{a}_{a}$	$4\ (0.0)^{a}_{a}$	$4\ (0.0)^{a}_{a}$	$4\ (0.0)^{a}_{a}$	$4\ (0.0)^{a}_{a}$	
	40	$1\ (0.1)^{a}_{b}$	$2\ (0.8)^{b}_{b}$	$4\ (1.0)^{c}_{a}$	$4\ (1.0)^{c}_{a}$	$2\ (0.9)^{d}_{b}$	
Overall preference***	0	1	0	1	1	1	16^{****}
	40	1	3	4	9	3	0^{****}

* Values represent the scale of overall acceptability (9 to 1) and the mean and standard deviation (in parentheses) of 20 observations. Means within each row with different superscripts are significantly (P<0.05) different. Means within each column with different subscripts are significantly (P<0.05) different.
** Values represent the scale of colour (4 to 1). Values are the mean and standard deviation (in parentheses) of 20 observations. Means within each row with different superscripts are significantly (P<0.05) different. Means within each column with different subscripts are significantly (P<0.05) different.
*** Number of panellists out of 20 who expressed their preference for the particular sample.
**** Number of panellists out of 20 who did not distinguish any differences.

erence for salad with rosemary at a concentration of 400 ppm. The salad without antioxidant had developed a rancid off-flavour. Caffeic acid, carnosol, carnosic and rosmarinic acid and other polyphenols present in extract have been reported to have good antioxidant action (alone or synergistically) (SHAHIDI, 2000; CUVELIER *et al.*, 1996) and, obviously, protected the product from oxidative deterioration.

Setting 10 hours as the end limit of the induction period, after which the product had unacceptable sensory properties, an estimation of the shelf-life of Tarama salad could be made using the linear equation. The theoretical decrease in the induction period after the 40th day until the induction period became 10 hours was calculated using the linear equation Y = a + bX (where Y = induction period and X = number of days) taking into account the intercepts and slopes of each batch. The calculated values were Y=38.797-0.477X (salad without antioxidant), Y=37.73-0.218X (salad with 100 ppm of rosemary extract), Y=40.402-0.222X (salad with 200 ppm of extract), Y=41.325-0.205X (salad with 400 ppm of extract) and Y=38.679-0.219X (salad with 200 ppm BHA). According to the above equations, the number of days before the induction period of each batch of salad reached the value of 10 hours (Y=10) was calculated to be 102.3 (without antioxidant), 127.2 (100 ppm of extract), 139.9 (200 ppm of extract), 152.8 (400 ppm of extract) and 131.1 (200 ppm of BHA). All antioxidants increased the shelf-life of the product. The rosemary extract at 400 ppm (increase 49%) was the most effective followed by the same extract at 200 ppm (34%) and BHA (28%), while the rosemary extract at 100 ppm increased the shelf-life the least (24%).

The rosemary extract at 400 ppm gave the highest protection against oxidation during the 40 days of storage and the longest shelf-life to the salad (as calculated by the linear equation) followed by the 200 ppm concentration. However, the same extract at 100 ppm showed the least protection (significant at $P<0.05$) during the 40 days of storage. The artificial antioxidant (BHA) showed moderate protection and extended the shelf-life of the product less than the rosemary extracts at 200 and 400 ppm but more than the extract at 100 ppm. Therefore, the natural antioxidant appears to be a good substitute for the synthetic one (BHA) at concentrations of 400 and 200 ppm. The results reported by ESTEVEZ *et al.* (2007) and SEBRANEK *et al.* (2005), regarding use of rosemary essential oils or extract to protect against the oxidative deterioration of liver paté and pork sausage, are in agreement with our results. In most cases, the natural antioxidant activity was proved superior to that of the artificial ones (BHT and BHA). However, these authors used substantially higher concentrations (1,000 to 2,500 ppm) of rosemary extract or essential oil. Other authors (CHE MAN and TAN, 1999) have also reported that rosemary extract had a higher antioxidant activity than BHA and BHT. Since Tarama salad is kept in the fridge, the addition of an antioxidant delays the oxidation of the lipid phase and prolongs the shelf-life of the product.

Deples

Table 3 illustrates the changes in the induction period of the oil extracted from Deples. The antioxidant improved the resistance to oxidative rancidity. The decrease in the induction period was significant ($P<0.05$) in all cases. The rosemary extract at a concentration of 150 ppm gave the highest protection (significant at $P<0.05$) to the product followed by the extract at a concentration of 100 ppm. The polyphenolic composition is responsible for the higher protection of the extract.

Setting 1 hour as the end limit of the induction period after which the product

Table 3 - Changes in induction period* of the lipid phase extracted from Depleses samples with rosemary extract during 35 days of storage.

Day	No antioxidant	Extract 50 ppm	Extract 100 ppm	Extract 150 ppm
0	26.1 (1.1)$^{a}_{a}$	31.9 (1.8)$^{b}_{a}$	34.3 (1.7)$^{c}_{a}$	37.3 (1.3)$^{d}_{a}$
7	22.2 (0.8)$^{a}_{b}$	27.0 (1.1)$^{b}_{b}$	30.2 (1.2)$^{c}_{b}$	35.2 (1.5)$^{d}_{b}$
14	15.2 (0.7)$^{a}_{c}$	20.7 (1.3)$^{b}_{c}$	25.1 (0.8)$^{c}_{c}$	30.4 (0.9)$^{d}_{c}$
21	10.1 (0.9)$^{a}_{d}$	15.2 (0.8)$^{b}_{d}$	20.8 (1.1)$^{c}_{d}$	25.6 (1.1)$^{d}_{d}$
28	4.5 (0.7)$^{a}_{e}$	10.3 (0.9)$^{b}_{e}$	15.8 (1.0)$^{c}_{e}$	22.5 (1.1)$^{d}_{e}$
35	1.5 (0.7)$^{a}_{f}$	5.1 (0.6)$^{b}_{f}$	10.9 (1.3)$^{c}_{f}$	20.2 (0.7)$^{d}_{f}$

* Values are the mean and standard deviation (in parentheses) of triplicate determinations of three replicate experiments carried out simultaneously (nine determinations in total). Means within each row with different superscripts are significantly (P<0.05) different. Means within each column with different subscripts are significantly (P<0.05) different.

has unacceptable sensory properties, an estimation of the shelf-life of Depleses can be made using the linear equation. The equations were Y=26.949-0.7396X (no antioxidant), Y=32.683-0.7739X (50 ppm of extract), Y=35.271-0.6714X (100 ppm of extract) and Y=38.229-0.5241X (150 ppm of extract). According to the above equations, the number of days before the induction period of each batch reached the value of 1 hour (Y=1) were calculated as 35.1 (no antioxidant), 40.9 (50 ppm), 51.0 (100 ppm) and 71.0 (150 ppm). The greatest increase was achieved with the rosemary extract at 150 ppm (102.3% increase) followed by 100 ppm (45.3%) and 50 ppm (16.5%).

The inhibition of oxidation of the frying oil absorbed by Depleses is very important. The oil continues to oxidize during storage which results in an unacceptable and unhealthy product. These results are in accordance with those of the sensory evaluation after 35 days of storage (Table 4). The overall acceptability as expressed by the score 9 to 1 was equal in all cases (not significant at P<0.05) at Day 0. According to Table 4 Depleses were generally acceptable with or without antioxidant since there was no

Table 4 - Sensory scores of Depleses.

Sensory attributes	Day	No antioxidant	Extract 50 ppm	Extract 100 ppm	Extract 150 ppm	
Overall acceptability*	0	9 (0.0)$^{a}_{a}$	9 (0.0)$^{a}_{a}$	9 (0.0)$^{a}_{a}$	9 (0.0)$^{a}_{a}$	
	35	4 (1.0)$^{a}_{b}$	6 (2.0)$^{b}_{b}$	8 (1.1)$^{c}_{b}$	8 (1.0)$^{c}_{b}$	
Colour**	0	1 (0.0)$^{a}_{a}$	4 (0.0)$^{b}_{a}$	4 (0.0)$^{b}_{a}$	4 (0.0)$^{b}_{a}$	
	35	1 (0.0)$^{a}_{b}$	4 (0.1)$^{b}_{a}$	4 (0.2)$^{b}_{a}$	4 (0.1)$^{b}_{a}$	
Overall preference***	0	2	2	1	1	14****
	35	0	2	5	12	1****

* Values represent the scale of overall acceptability (9 to 1) and are the mean and standard deviation (in parentheses) of 20 observations. Means within each row with different superscripts are significantly (P<0.05) different. Means within each column with different subscripts are significantly (P<0.05) different.
** Values represent scale of colour (4 to 1) and the mean and standard deviation (in parentheses) of 20 observations. Means within each row with different superscripts are significantly (P<0.05) different. Means within each column with different subscripts are significantly (P<0.05) different.
*** Number of panellists out of 20 who expressed their preference on the particular sample.
**** Number of panellists out of 20 who did not distinguish any differences.

statistically significant difference in total acceptability at Day 0. However, after 35 days of storage Deples containing 100 and 150 ppm of rosemary extract were the most acceptable (significant at $P<0.05$) among the whole lot of products. In evaluating the overall preference, the panellists showed a preference for Deples containing antioxidant after 35 days of storage compared to Deples without antioxidant. Rosemary extract, at all concentrations, significantly ($P<0.05$) reduced the darkening of Deples while the product without antioxidant turned brown. According to LALAS and DOURTOGLOU (2003), the colour of fried products (potato chips) is produced mainly by Maillard reactions. Antioxidants not only reduce oxidation but also, indirectly block the Maillard reaction by inhibiting of the final pyrazine formation phase (PORTER *et al.*, 2006). This reduces the formation of the dark colour that occurs as a result of reactions between free sugars and amino acids during frying, especially in the present case where orange juice (high sugar content) was used in the recipe. Finally, a pungent taste (due to the oxidation of the absorbed oil in Deples), made the product unacceptable by the panellists, especially after 35 days of storage (0 in overall preference) (Table 4).

In conclusion, rosemary extract protected Tarama Salad and Deples against oxidation and extended their shelf-life. The use of rosemary extract has a positive effect on consumer acceptance (as indicated by the sensory evaluation) of all the products that contain a considerable amount of oil (absorbed during frying or added during preparation).

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