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The influence of different oils on the death rate of *Salmonella enteritidis* in homemade mayonnaise

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The death rate of *Salmonella enteritidis* was always faster in mayonnaise made with extra virgin olive oil than in that prepared from blended olive or sunflower oils. The acidity and the phenolic profiles of these oils differed significantly. The most acidic oils (0·5% oleic acid), the extra virgin oils, also had the most complex phenolic profiles. The acidity of sunflower and blended olive oil was 0·2% and 0·4% respectively.

Mayonnaise, an oil-in-water emulsion, is made from vegetable oils, egg yolk, water, vinegar and/or lemon juice, sodium chloride and, depending upon recipe, sweeteners, spices or solium glutamate (Smittle 1977). Mayonnaise and related products are unlikely to transmit food-poisoning bacteria because of their low pH values, of about 4·1 (Collins 1985). Wethington & Fabian (1950) and Perales & Garcia (1990) showed that the numbers of salmonellas and staphlococci diminished rapidly at values below 4·1, the duration of the survival period being determined by the concentration of acetic or citric acid. Low temperature, and low a_w values in mayonnaises may inhibit foodborne pathogens (Smittle 1977). Products containing less than normal amounts of acid have been associated with foodborne outbreaks of salmonellosis and *Staphylococcus aureus* (Collins 1985; Mitchell *et al.* 1989; Gomez-Lucia *et al.* 1990; Perales & Garcia 1990).

Commercial edible oils differ in their acidity (Kiritsakis 1988) but, according to Wethington & Fabian (1950), such differences are unlikely to effect appreciably the microbial stability of mayonnaise. The present paper provides evidence that different grades of oil do influence the death rate of salmonellas in mayonnaise.

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Materials and Methods

MICROBIOLOGICAL ANALYSIS

Mayonnaise preparation

Mayonnaise (300 ml oil; 2 egg yolks (size 2); 9 ml (6% v/v) acetic acid; final pH 4·3) was prepared by whisking two egg yolks with an electric hand mixer (Carlton AMO3) and gradually adding the oil (150 ml) with a sterile pasteur pipette during continuous mixing. Six ml of acetic acid were whisked in to thin down the mixture so that it did not curdle. The remaining oil (150 ml) was then added and finally another 3 ml of acetic acid. The final pH, measured with a pH meter (EIL 7050), was 4·3. The following oils were used: sunflower, a proprietory blend of olive oils from EEC countries, and extra virgin olive oils from Italy and Greece.

Organism

Salmonella enteritidis strain PT4, kindly provided by Dr T. Humphrey (Exeter PHLS), was

maintained on Nutrient agar (lab m) slopes at 4°C and subcultured weekly. An overnight culture was grown in 50 ml nutrient broth (lab m) at 37°C, harvested by centrifugation, washed twice in saline and resuspended in saline to give a cell density of 10^9 cells/ml.

Inoculation

About 5×10^4 cfu/g *Salm. enteritidis* PT4 were throughly mixed with 40 g of mayonnaise by extensive stirring with a spatula. Uninoculated samples were used as controls. Samples were incubated at 20°C. The experiments were done four times with seven replicates per oil on one occasion and four in a further three trails.

Bacterial enumeration

At 0, 24, 48 and 72 h samples were diluted in 1/4 Ringer solution, 0·1 ml of an appropriate dilution was spread on nutrient agar in Petri dishes and colonies counted after 24 h at 37°C.

CHEMICAL ANALYSIS

Extraction of phenolic compounds

Oil (30·00 + 0·01 g) diluted in 50 ml hexane was extracted with 3×50 ml of methanol : water (60 : 40 *v/v*)—thorough mixing was achieved with a Kenwood chef mixer (Graciani Constante & Vazquez Roncero 1980). Two fractions were separated by centrifugation ($300 \times$ **g** for 10 min). The aqueous-alcoholic fractions from each extraction were filtered through damp Whatman No. 1 filter paper and evaporated under vacuum at 35–40°C. The residue was disolved in 2 ml methanol, filtered through a millipore filter (0·2 μm) and used for HPLC.

HPLC

The HPLC analyses were done by the method of Tassou *et al.* (1991). Oleuropein (Extrasynthex, France), and tyrosol, rutin, vanillic acid, caffeic acid and 3,4-dihyroxyphenylacetic acid (Aldrich Chemical Co. Ltd) were used for reference. All the standards were diluted in methanol and analysed by HPLC in the same way as those from the oils.

Determination of acidity

The method of Kiritsakis (1988) was used. An oil sample (28·2 g) in an Erlenmeyer flask (250 ml) was mixed with 50 ml ethanol (95% v/v) and 2 ml phenolphthalein (1% w/v in 95% ethanol) and titrated slowly with 1N NaOH until a pink colour appeared. The acidity was estimated thus: acidity (% oleic acid) = ml NaOH × Molarity NaOH × 0·282* × 100 × g^{-1} (*: 0·282 = mg of oleic acid).

Results

MICROBIOLOGICAL ANALYSIS

The death rates of salmonellas in mayonnaise made with virgin olive oil (Greek or Italian) were faster than in those containing blended olive or sunflower oil (Table 1). No viable *Salm. enteritidis* were recovered from mayonnaise made with virgin oil after 72 h incubation but they were present in those made with blended or sunflower oil and incubated at 20°C for 3 d (Fig. 1). When all four experiments are considered (Table 1), the mayonnaise made from sunflower was always the least toxic. On two occasions the death rate of *Salm. enteritidis* in mayonnaise made from blended oil was not significantly different from that containing sunflower but on two occasions the former was faster than the latter. In all cases the death rates in mayonnaise made from extra virgin oil were significantly faster than those containing sunflower or blended oils.

Table 1. The effect of different vegetable oils on the death rate of *Salmonella enteritidis* PT4 in mayonnaise incubated at 20°C

	Experiment			
Oil used	1	2	3	4
F-test*	***	***	***	***
Sunflower	x†	x	x	x
Blend of olive oils	x	y	x	y
Italian olive oil (virgin)	y	ND	y	ND
Greek olive oil (virgin)	y	z	y	z

*** Significant at 0·1% probability level.

† Means with the same letter did not differ significantly. ND, Not determined.

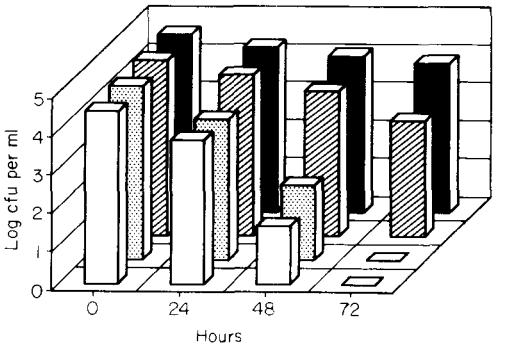

Fig. 1 The death rate of *Salmonella enteritidis* in mayonnaise made with different oils at 20°C. □, Italian extra virgin olive oil; ▨, Greek extra virgin olive oil; ▨, blended olive oil; ■, sunflower oil.

ANALYSIS OF OILS

There were significant differences ($P < 0{\cdot}1\%$) in the acidity of oils used—viz. sunflower (0·2%; least acid), blended olive oil (0·4%) and the two extra virgin oils (0·5%; most acid)—as well as their range of phenolic compounds. The blended olive oil and sunflower oil contained insignificant amounts of such compounds whereas the extra virgin olive oils contained the greatest range (Fig. 2). Tyrosol occurred at different concentrations (peak 3; Fig 2) and oleuropein (peak 10) only in the virgin olive oils, its concentration being greatest in that of Greek origin. This oil contained phenolic substances identified with caffeic acid (peak 4), rutin (peak 6) and vanillic acid (peak 7). Traces of protocatechuic acid (peak 2a) occurred only in the Italian virgin olive oil. Two unidentified peaks (2 and 11) were presented in the virgin olive oil of both origins. Traces of 3,4-dihydroxyphenylacetic acid (peak 2b) were identified only in the blended olive oil.

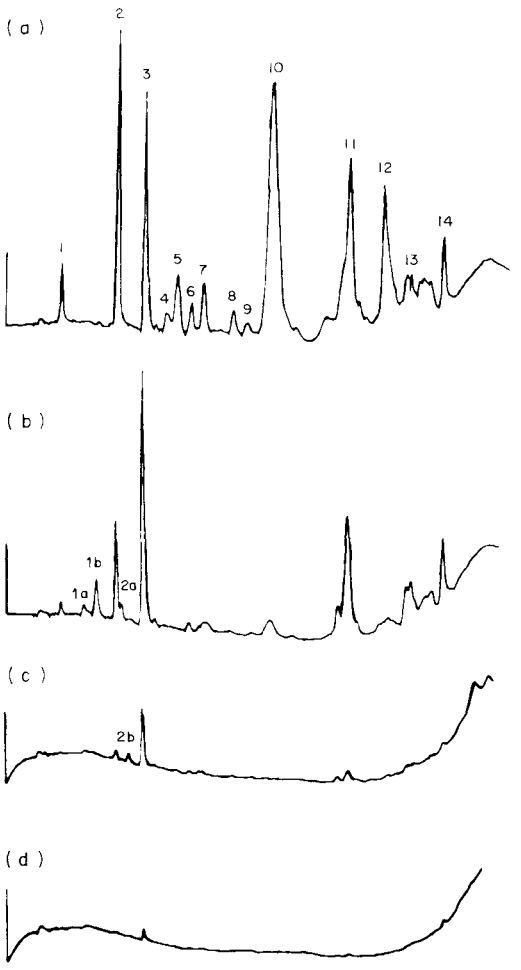

Fig. 2. The profiles of the phenolic compounds in (a) Greek extra virgin olive oil, (b) Italian extra virgin olive oil, (c) blended olive oil, and (d) sunflower oil. Peaks 2a, 2b, 3, 4, 6, 7 and 10 were identified with protocatechuic acid, 3,4-dihydroxyphenylacetic acid, tyrosol, caffeic acid, rutin, vanillic acid and oleuropein respectively; the rest of the peaks were unidentified.

Discussion

In systems such as mayonnaise there is a greatly expanded oil/water interface which exerts a marked influence on the distribution of long-chain free fatty acids and phenolics between the aqueous and oil phases (Cornell 1979; Collins 1985). It is well known, also, that long- and/or short-chain fatty acids as well as phenolic compounds act as antimicrobial agents on foodborne bacteria (Branen *et al.* 1980; Nychas *et al.* 1990; Tassou *et al.* 1991). These substances could well affect the fate of foodborne bacteria in mayonnaise. Edible oils contain both phenolic and long-chain fatty acids in various concentrations. The concentration of the long-chain fatty acids was the main difference between olive and sunflower oil. Although oleic (18 : 1) and linoleic (18 : 2) acids are present in both oils, the former is dominant in olive and the latter in sunflower oil (Kiritsakis 1988). In this study the acidity (free fatty acids) in the two virgin olive oils was higher than in the sunflower oil.

The type and the concentration of phenolic compounds found in the oils differed significantly (Fig. 2). These differences presumably reflect the extraction methods, the variety of olives used for extractions as well as the period and the storage history of the oil (Kiritsakis 1988). In general olive oil extracted by mechanical methods contains phenolic compounds in the range of 50–157 ppm whilst solvent-extracted oils contain 321–574 ppm (Kiritsakis 1988). The presence of the phenolic compounds identified from Greek or Italian virgin olive oil in this study has been reported by other workers also (Solinas *et al.* 1975; Graciani Constante & Vazquez Roncero 1980). It needs to be noted, however, that emphasis has usually been given to the antioxidant activity of the olive oils, or to their contribution to organoleptic properties rather than their antimicrobial activity. The latter attribute was emphasized in the present study.

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