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Effect of extracts of oak (*Quercus petraea*) bark, oak leaves, aloe vera (*Curacao aloe*), coconut shell and wine on the colloidal stability of milk and concentrated milk

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Abstract

Addition of aloe vera extract, non-dialyzable red wine residue or aqueous methanol extracts of oak bark, oak leaves or coconut shell increased the heat stability of skim milk (at 140°C) and concentrated milk (at 120°C) and retarded rennet coagulation, but had no effect on the alcohol stability of milk. The calcium ion concentration in milk was reduced by the addition of these extracts but calcium chelation does not appear to be the exclusive mechanism responsible for promoting micellar stability. The extracts contained a high concentration of polyphenols, which are highly reactive and may be the active agents in the extracts, responsible for the enhanced stability of casein micelles. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The colloidal stability of casein micelles is maintained, among other factors, by steric stabilisation, a negative zeta potential (ca. −20 mV) and hydration and is destabilised by heat, alcohols, enzymatic hydrolysis and acidification (Schmidt & Payens, 1976; Horne, 1986). Reduction of micellar stability is desirable in the production of some products, e.g. yoghurt and cheese (Dargan & Savello, 1990; Fox, 1993). However, the thermal stability of milk, especially concentrated milk, is limiting and considerable efforts have been made to develop stabilisers, e.g. lecithin, urea, calcium chelators, etc, or procedures which promote heat stability, e.g. preheating and pH adjustment (Muir & Sweetsur, 1976; Fox & Hearn, 1978; Holt, Muir, & Sweetsur, 1978; Fox, 1982; Shalabi & Fox, 1982; Singh & Fox, 1985). (For general reviews on the heat stability of milk, see Fox & Morrissey, 1977; Fox, 1982; van Boekel, Nieuwenhuijse, & Walstra, 1989; Horne & Muir, 1989; Singh & Creamer, 1992; McCrae & Muir, 1995).

O'Connell, Fox, Tan-Kintia, and Fox (1998) reported that extracts of green or black tea, cocoa powder or coffee markedly increased the heat stability and rennet coagulation time of milk; all these extracts contain a high level of polyphenols. The communication reports the results of preliminary studies on the effect of extracts from other polyphenol-rich materials, i.e. red wine, oak bark, oak leaves, aloe vera and coconut shell, on the heat stability, rennet coagulability and ethanol stability of milk and concentrated milk.

2. Materials and methods

2.1. Milk supply

Raw milk, obtained from a local dairy, was defatted by centrifugation at 2000 ***g*** for 20 min at 20°C, followed by filtration through glass wool to remove fat particles. The skimmed milk was stored at 4°C until required (not more than 3 days).

Concentrated milk was prepared by dissolving low-heat skimmed milk powder (Irish Dairy Board, Dublin) in distilled water to 22.5% total solids.

Rennet coagulation time (RCT) was determined on fresh skimmed milk or skim milk prepared by reconstituting low-heat skimmed milk powder (Station de Recherches en Technologie et Analyses de Laitieres, Poligny, France) in H_2O to 9.0% TS, with and without addition of $CaCl_2$ to 4.5 mM.

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2.2. Preparation of extracts from wine, coconut, oak leaves and oak bark

Red wine (700 ml, Valpolicella, Simbottigliato da Salvalai A & C SpA, Bagnolo Mella, Italy) was placed in T 88-108 dialysis tubing (Medicell, London, UK) and dialysed against ca. 15 l of H_2O at 4°C for 60 h with 6 water changes. The retentate was freeze-dried, yielding ca. 1 g of powder which is hereafter referred to as non-dialyzable red wine residue (NDRWR).

Coconut shells (300 g, Copper Glow Ltd, Dublin, Ireland) were pulverised in a Waring blender and the powder extracted with 4 l of chilled 50% aqueous methanol for 1 h, with occasional stirring. After incubation, the extract was filtered through glass wool, followed by filtration through Whatman No. 113 filter paper under vacuum. The methanol was removed by using a rotary evaporator under vacuum at 35°C. The residue was freeze-dried, yielding ca. 18 g of powder which was stored in a desiccator.

Dead oak leaves (100 g) were obtained from a young tree (ca. 20 years), cut into strips and pulverised in a Waring blender. The powder was extracted with 2 l of chilled 50% aqueous methanol for 1 h, followed by filtration through glass wool and Watman No. 113 filter paper under vacuum. The methanol was removed using a rotary evaporator at 35°C under vacuum. The residue was freeze-dried, yielding 1.8 g of powder.

Oak bark (100 g) was extracted as for oak leaves except that the incubation period was preceded by sonication for 10 min (ultrasonic bath, model U100, Ultrawave Ltd, Cardiff, UK); 100 g of bark yielded ca. 8.6 g of powdered extract.

2.3. Aloe vera extract

Aloe vera extract, under the name 'Aloin', containing ca. 20%, w/w, barbaloin (10-glucopyranosyl-1,8-dihydroxy-3-[hydroxy-methyl]-9[10H]-anthracenone) was purchased from Sigma Chemicals Co. (St Louis, MO, USA).

2.4. Influence of extracts on some physiochemical properties milk

Extracts were added at 0.4% (w/v) to standard milk and at 0.8% (w/v) to concentrated milk and stirred for 30 min. The heat stability, rennet coagulation time, alcohol stability and calcium ion concentration of samples of control and experimental milks were determined as methods described by O'Connell et al. (1998).

2.5. Determination of total concentration of phenolic compounds in extracts

Samples of the dried extracts were dissolved in H_2O at 0.4% (w/v). Aliquots (40 µl) of the extracts were placed in screw-capped test tubes, 500 µl of Folin-Ciocalteu phenol reagent (Merck, Darmstadt, Germany) added and thoroughly mixed. Immediately, 5 ml of 20% (w/v) Na_2CO_3 were added and mixed vigorously. The samples were then centrifuged at 1200 ***g*** for 15 min at 20°C and absorbance read at 735 nm.

The total concentration of phenolic compounds was expressed as equivalents of gallate using a standard curve of gallate concentration against absorption at 735 nm (Julkunen-Tiito, 1985).

Note: Because of the subjective nature of the heat stability, rennet coagulation and alcohol stability assays, and the large effect of small differences in pH on the values obtained, statistical analysis of results of replicate experiments was not possible; all experiments were repeated at least 3 times and were reproducible, i.e. a similar effect was found in all cases.

3. Results and discussion

The effect of bark extract on the heat stability of unconcentrated milk is shown in Fig. 1; the effects of NDRWR, aloe vera and oak leaf extracts were essentially similar (results not shown). The extracts markedly increased stability, particularly in the region of the minimum, pH 6.8–7.1, i.e. converted a type A HCT–pH profile to a type B profile (Fox, 1982). Addition of coconut shell extract did not eliminate the minimum but did increase the stability of unconcentrated milk, principally by shifting the HCT–pH profile to more alkaline values, increasing stability at the maximum and diminishing the pH range over which the minimum occurred (results not shown).

The effect of bark extract when added at a pro rata concentration on the heat stability of concentrated milk prepared from low-heat skim milk powder is shown in Fig. 2. Addition of leaf, NDRWR, aloe vera or coconut

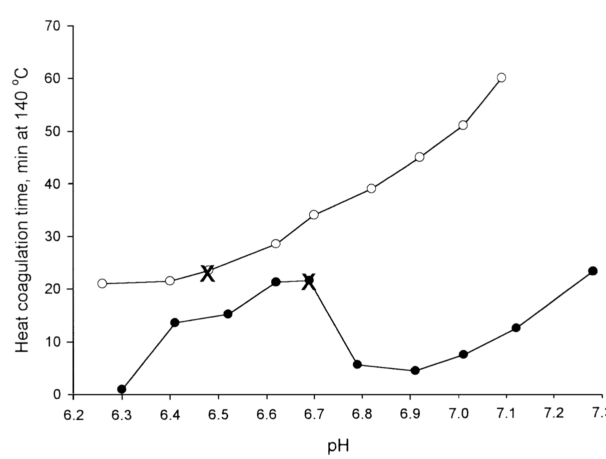

Fig. 1. Effect of 50% aqueous methanol oak bark extract at 0 (●) or 0.4% w/v (○) on the HCT–pH profile of skimmed milk; **X**, original pH.

shell extracts had a similar effect (results not shown), i.e. shifted the HCT–pH profile to more alkaline values and enchanced stability at the maximum.

The data in Table 1 show that the addition of bark, leave, aloe vera, NDRWR or coconut shell extracts increased the rennet coagulation time (RCT) of milk and reconstituted milk, especially when the latter was not supplemented with $CaCl_2$. Bark extract had the greatest effect on RCT, followed by aloe vera, wine, leaves and coconut shell, in decreasing order. This order of effectiveness is also evident in the ability of the extracts to increase heat stability, although the relative differences were smaller.

The addition of $CaCl_2$ to 4.5 mM reduced the RCT of both control and experimental milks, suggesting that the effect of the extracts was on the secondary phase of coagulation, i.e. aggregation of enzymatically modified micelles, rather than on the primary phase.

The third physiochemical property investigated was alcohol stability. None of the extracts affected the alcohol stability of milk throughout the pH range 6.4–7.2 (results not shown).

Calcium ion concentration affects the heat stability, rennet coagulation and alcohol stability of milk. All the extracts reduced the calcium ion concentration in milk, but to different extents (Table 2). The extracts prolonged RCT, irrespective of whether or not milk was supplemented with $CaCl_2$. The effect of bark extract on the heat

Table 2
Influence of extracts of wine, aloe vera, coconut shell, oak leaves or oak bark on the calcium ion concentration of milk at 21°C

System	Calcium ion concentration (mM)
Control	4.96
50% aqueous methanol extract of oak bark	3.80
50% aqueous methanol extract of oak leaf	4.20
50% aqueous methanol extract of coconut shell	3.80
Non-dialyzable red wine residue (NDRWR)	4.60
Aloe vera extract	3.86

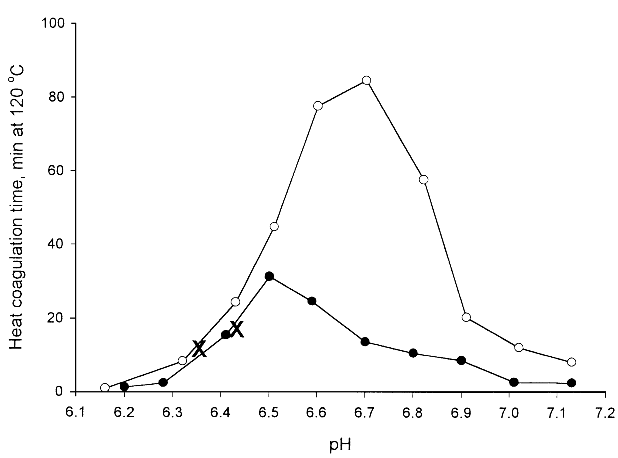

Fig. 2. Effect of 50% aqueous methanol oak bark extract at 0, (●) or 0.8% w/v (○) on the HCT–pH profile reconsituted low-heat skimmed milk powder (22.5% TS); **X**, original pH.

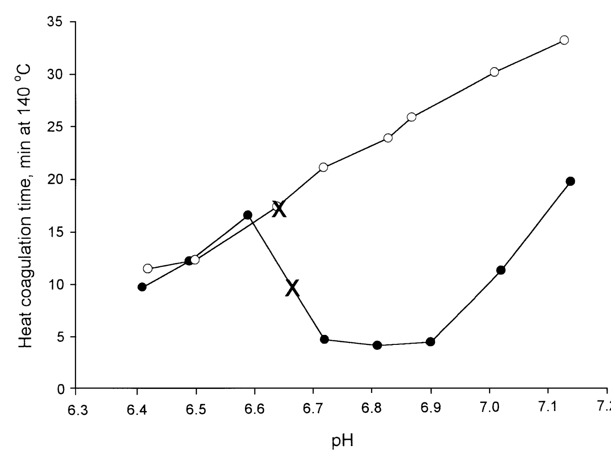

Fig. 3. Effect of dialysing skimmed milk (50 ml) containing 0, (●) or 0.4% w/v (○), 50% aqueous methanol extract of oak bark, against 2×4 l batches of bulk milk at 4°C for 48 h on the HCT–pH profile; **X**, original pH.

Table 1
Influence of extracts of wine, aloe vera, coconut shell, oak leaves or oak bark on the rennetability of fresh skimmed milk and reconstituted skimmed milk, with and without the addition of $CaCl_2$

System	Control	With bark extract at 0.4% (w/v)	With leaf extract at 0.4% (w/v)	With NDRWR[a] at 0.4% (w/v)	With aloe vera extract at 0.4% (w/v)	With coconut shell extract at 0.4% (w/v)
				RCT[b] (min)		
Milk	2.61 ± 0.13	7.42 ± 0.60	3.63 ± 0.15	3.73 ± 0.40	4.1 ± 0.01	3.13 ± 0.15
Reconstituted skim milk (9% TS) without $CaCl_2$	5.93 ± 0.12	> 30.00[c]	11.75 ± 1.0	17.33 ± 0.52	21.6 ± 0.20	8.70 ± 0.20
Reconstituted skim milk (9% TS) with $CaCl_2$	1.37 ± 0.21	4.87 ± 0.25	2.87 ± 0.12	5.1 ± 1.00	4.75 ± 0.15	2.23 ± 0.1

[a] NDRWR = non-dialyzable red wine residue.
[b] RCT = rennet coagulation time at 30°C.
[c] Did not coagulate within 30 min.

Table 3
Total phenolic content of wine, aloe vera, coconut shell, oak leaves or oak bark extracts as expressed mg gallate equivalent/g extract

Sample	Total phenolic content, mg gallate equivalents/g extract
50% aqueous methanol oak bark extract	313 ± 7.0
50% aqueous methanol oak leave extract	184 ± 0.3
50% aqueous methanol coconut shell extract	36.8 ± 4.3
Non-dialyzable red wine residue (NDRWR)	147 ± 1.3
Aloe vera extract	144 ± 2.0

stability of milk was not eliminated when milk containing bark extract was dialysed against bulk milk (50 ml against ~ 4 l bulk milk for 48 h at 4°C, with two changes of bulk milk, Fig. 3). These results suggest that the chelation of calcium is not primarily responsible for the stabilising effect of the extracts. This view is supported by the fact that none of the extracts had an effect on the alcohol stability of milk, which is very sensitive to [Ca^{2+}] (Horne & Parker, 1981).

O'Connell et al. (1998) suggested that the stabilizing effect of tea, coffee and cocoa extracts might be due to to polyphenols. All the extracts in the present study also contain polyphenols at concentrations ranging from 36–312 mg gallate equivalent/g. The effectiveness of the extracts was in the order of their polyphenol content; the extract of coconut shell which had the least effect on the heat stability and rennet coagulation had by far the lowest concentration of polyphenols.

Polyphenols are highly reactive compounds due principally to the fact that they are polydentate ligands with a multiplicity of potential binding sites, i.e., phenolic groups (Haslam & Lilley, 1988; Spencer et al., 1988). There have been many studies on the ability of polyphenols to chelate metals and interact with proteins (Radhakrish & Sivaprasad, 1980; Haslam & Lilley, 1988). Apart from a study by Brown and Wright (1963) on the effect of polyphenols on the electrophoretic mobility of milk proteins and a study by O'Connell et al. (1998) on the effect of polyphenol-rich extracts of tea, coffee and cocoa on the colloidal stability of milk, the effect of polyphenols on the physiochemical properties of milk proteins does not appear to have been studied and warrants further research.

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