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D.B. LUND
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INFLUENCE OF SODIUM PHOSPHATE ON THE HEAT STABILITY OF BUFFALO MILK AND ITS CONCENTRATE

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ABSTRACT

The influence of three different concentrations, 0.05%, 0.10% and 0.15% of monobasic sodium phosphate on the heat stability (at 130°C) and pH of buffalo milk and its 2:1 concentrate was determined. It was observed that sodium phosphate caused a considerable increase in the heat stability, determined as heat coagulation time (HCT) of concentrated buffalo milk. The optimum concentration of sodium phosphate for imparting maximum stability to the concentrate was different for different samples. However, with the addition of an appropriate concentration of sodium phosphate it was possible to manufacture evaporated milk up to 36% total solids. Depending on the HCT/pH profile, some of the samples of fluid (unconcentrated) milk were stabilized while the others were destabilized due to the addition of sodium phosphate. Addition of monobasic sodium phosphate caused a decrease in the pH of fluid milk and its concentrate.

INTRODUCTION

The instability of buffalo milk to heating, particularly when it is concentrated and sterilized (as in the manufacturing of evaporated milk) poses considerable problems (Srinivasan *et al.* 1967) and so far has prevented the manufacturing of evaporated milk from it. Roy and Yadav (1972) reported the possibility of manufacturing evaporated milk from buffalo milk with the addition of 1% acid casein. A study in this laboratory (Tayal and Sindhu 1983) revealed that while the addition of acid casein increased the heat stability of concentrated buffalo milk the increase was not sufficient to enable the concentrate to withstand the sterilization temperature. Stabilizing salts such as sodium phosphate and sodium citrate have been successfully used to correct the salt balance of bovine milk to give increased stability to its evaporated products (Verma 1965). However, recent studies in our laboratory (Tayal 1983; Sindhu and Tayal 1984) revealed that disodium phosphate and sodium citrate acts as destabilizing agents when added to buffalo milk prior to its concentration. This

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destabilization may be attributed to an increase in milk pH per se. Since the pH was in the range of minimum stability in the heat stability/pH profile (maximum stability of concentrated buffalo milk was found in the acidic side (pH 6.5-6.6) of normal pH, 6.6-6.7). The investigation described in this communication was carried out to determine the influence of monobasic sodium phosphate (which cause a decrease in pH) on the heat stability of buffalo milk and its 2:1 concentrate.

MATERIALS AND METHODS

The pooled milk samples were collected from the Institute herd of Murrah buffaloes during morning milking. Each sample was divided into four lots. Monobasic sodium phosphate ($NaH_2PO_4H_2O$) was added at levels of 0-0.15% and samples heated at 85°C for 5 min. After cooling the heated milk to room temperature a portion was taken from each lot for the determination of heat-stability and the remainder concentrated at 55 ± 1°C in a rotatory type vacuum evaporator at an absolute pressure of 0.3 mm H_g.

The heat stability of all milks was determined at 130°C ± 1°C as previously reported (Tayal and Sindhu 1983).

Statistical Analyses

Statistical analyses of the data for standard errors and F test was carried out according to Snedcor and Cochran (1968).

RESULTS AND DISCUSSION

Effect on Fluid Milk

The effect of addition of sodium phosphate on the heat stability determined as heat coagulation time (HCT) at 130°C and pH of buffalo milk are presented in Table 1. Results indicated that both the HCT and pH were affected by the addition of sodium phosphate. A decrease in milk pH which was progressive with the increase in the concentration of added phosphate was observed. However, no uniform trend was observed in the case of HCT. It is evident from the results in Table 1 that NaH_2PO_4 has a stabilizing effect in majority of the samples (samples 1-8). However, continuous increase in the concentration of added salt did not increase the stability of milk linearly. In the case of few samples (9-10) addition of Na_2HPO_4 has a destabilizing effect. As indicated in Fig. 1 this differential behavior of different samples was dependent on the HCT/pH profile of a particular

Table 1. Influence of added sodium phosphate (monobasic) on the heat coagulation time (HCT) at 130°C and pH of buffalo milk

Sample No.*	Concentration of added sodium phosphate (g/100 ml milk)							
	Nil		0.05%		0.10%		0.15%	
	pH	HCT	pH	HCT	pH	HCT	pH	HCT
1	6.80	30.55	6.70	46.00	6.60	48.33	6.50	49.50
2	6.80	27.00	6.70	42.50	6.60	44.50	6.50	42.10
3	6.80	33.00	6.70	34.00	6.60	38.00	6.55	35.00
4	6.80	34.00	6.65	36.00	6.60	39.00	6.55	40.00
5	6.80	54.00	6.60	59.00	6.50	68.00	6.45	75.00
6	6.75	30.00	6.65	31.00	6.55	32.30	6.40	34.30
7	6.70	38.70	6.60	45.70	6.50	50.75	6.35	49.00
8	6.80	39.00	6.70	39.10	6.60	40.00	6.50	42.00
9	6.80	44.50	6.70	43.40	6.60	43.00	6.55	40.20
10	6.80	45.00	6.70	43.30	6.60	42.00	6.45	41.00

* Each sample denotes a different day and was collected from the pooled milk of the institute herd of Murrah buffaloes during morning milking. The mean of HCT values was not calculated as it was irrelevant (since HCT decreased in some samples while in others it increased due to addition of sodium phosphate).

sample of milk. In the present investigation two types of HCT/pH profiles were observed in the case of buffalo milk (all the samples exhibited only type A characteristics). Both of these types of HCT/pH profiles are shown in Fig. 1. The majority of the samples (Samples 1-8, Table 1) had the pH of maximum stability on the acidic side of normal pH (maximum stability was observed at pH 6.7 while the normal pH was 6.8) and were stabilized by addition of NaH_2PO_4. In the case of sample 9 and 10 the maximum stability was observed at normal pH and these samples were destabilized due to the addition of sodium phosphate.

The stabilizing or destabilizing influence of sodium phosphate may be attributed definitely to its effect on pH. Those samples which had maximum stability at a pH which was in the acidic side of the normal pH, were stabilized by its addition because the decreased pH (6.5-6.7) was in the range of maximum stability. On the other hand, those samples which had

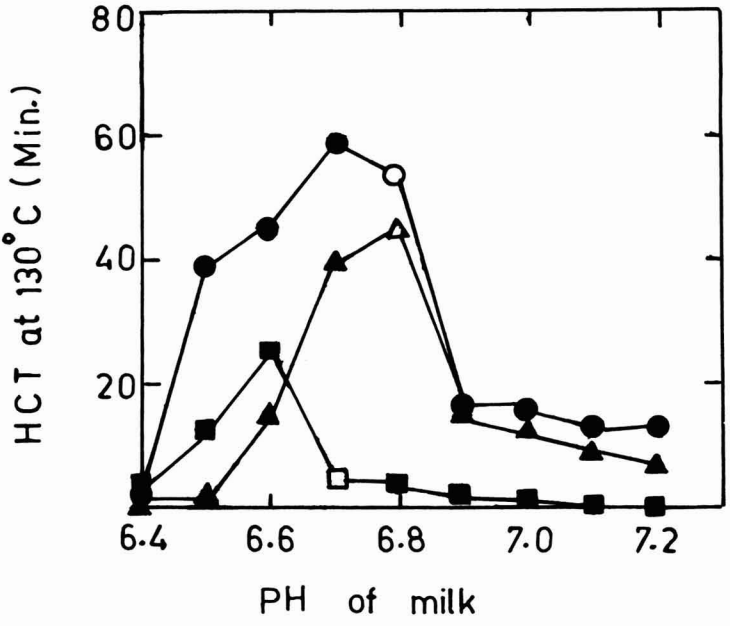

Fig. 1. HCT/pH PROFILE OF BUFFALO MILK AND ITS 1:2 CONCENTRATE

- ● Buffalo milk having maximum HCT in the acidic range of pH (Sample 1-8 Table 1).
- ▲ Buffalo milk having maximum HCT at normal pH (Sample 9-10 Table 1).
- ■ Concentrated buffalo milk.
  Open symbols denote the unadjusted pH.

maximum stability at normal pH were destabilized due to its addition because the decreased pH was in the range of minimum stability. Sweetsur and Muir (1980) also observed somewhat similar behaviour when NaH_2PO_4 was added to type A bovine milk; milk samples having normal pH in the alkaline side of the pH of maximum stability in the HCT/pH profile were stabilized while those which had the normal pH in the acidic side of the pH of maximum were destabilized by the addition of monobasic sodium phosphate.

Effect on Concentrated (1:2) Milk

The effect of sodium phosphate on the pH and HCT of the 2:1 concentrate are given in Table 2. While the statistical analyses for analyses of variance (F test) is given in Table 3. The pH of concentrated milk containing added sodium phosphate was significantly lower than that of the control (no added phosphate). Addition of monobasic sodium phosphate to fluid milk up to the level of 0.10% (0.2% of concentrate) caused a significant increase in the heat stability of concentrate. Further increase in the concentration of added sodium phosphate to 0.3% of the concentrate caused a decrease in the heat stability. In some of the samples due to the decrease the heat stability of concentrate containing 0.3% added salt was even lower than that of control. This increase in the HCT was due to addition of 0.1 to 0.2% sodium phosphate and was observed in all the samples of the concentrated milk irrespective of their HCT/pH profile before concentration. The reason for such an increase in the HCT of concentrated milk prepared from milk containing added sodium phosphate may be attributed to a decrease in its pH to the level at which the HCT was higher than the normal pH (unlike fluid milk all the samples of concentrated milk were found to have only one type of HCT/pH profile in which the pH of maximum stability was in the acidic side of the normal pH as shown in Fig. 1). The increase in the HCT of concentrated milk obtained with the addition of acid casein (Tayal and Sindhu 1983) may also be the result of a decrease caused in the pH.

The optimum concentration of sodium phosphate for maximum stability in the concentrate varied from sample to sample. In some cases 0.1% NaH_2PO_4 was sufficient while in others 0.2% and still in two of the samples 0.3% of the concentrate was required. The maximum limits of the stabilizers permitted by law in India is 0.3%. Therefore, on the basis of the results obtained in the present study it was not possible to recommend an exact concentration of sodium phosphate to give maximum stability to the concentrated milk. However, addition of 0.05% to 0.10% sodium phosphate (monobasic) to milk before its 2:1 concentration (to achieve 0.10 to 0.2% concentration of the salt in the finished product) resulted in an increase in

Table 2. Influence of added sodium phosphate (monobasic) on the heat stability (at 130°C) of concentrated (2:1) buffalo milk

Sample No.*	Concentration of added phosphate (g/100 ml concentrated milk)							
	Nil		0.10%		0.20%		0.30%	
	pH	HCT	pH	HCT	pH	HCT	pH	HCT
1	6.70	6.00	6.60	20.10	6.50	13.10	6.40	3.50
2	6.70	4.70	6.60	15.00	6.50	22.30	6.40	16.40
3	6.65	8.50	6.65	15.20	6.45	14.15	6.40	5.25
4	6.70	4.00	6.60	8.50	6.45	21.00	6.35	26.00
5	6.70	6.00	6.55	27.50	6.45	10.50	6.40	2.00
6	6.70	10.30	6.50	17.45	6.40	12.00	6.35	4.20
7	6.60	2.00	6.50	2.30	6.40	4.20	6.30	7.00
8	6.75	3.00	6.60	13.00	6.50	20.35	6.40	9.00
9	6.75	4.90	6.60	15.00	6.50	22.00	6.40	16.40
10	6.60	6.30	6.55	20.10	6.45	15.00	6.40	10.00
Average	6.70 ±0.050	5.57 ±0.741	6.55 ±0.0252	15.42 ± 2.041	6.45 ± 0.016	15.46 ± 1.776	6.40 ±0.0216	9.98 ±2.261

Table 3. Analysis of variance for the effect of monobasic sodium phosphate on the pH and heat stability (HCT) of concentrated buffalo milk[a]

Source of variation	df	SS		MSS		F	
		pH	HCT	pH	HCT	pH	HCT
Between samples	9	0.040	370.6256	0.00466	7.850	6.3**	1.77 NS
Between Treatments	3	0.508	684.5538	0.1693	228.19	22.88**	51.39**
Error	27	0.020	930.00	0.00074	34.440	--	--

[a] — Analysis is for the data reported in Table 2
df — Degrees of freedom
SS — Sum of Squares
MSS — Mean sum of squares
F — Variance ratio
NS — Not significant
** — Significant at P $<$ 0.01

the heat stability which was statistically significant and sufficiently high in most of the cases to enable the concentrate to withstand sterilization at 120°C for 10 min. With the addition of an appropriate concentration (from 0.05 to 0.15%) of sodium phosphate to milk before its concentration it was possible to prepare evaporated milk up to 36% total solids from buffalo milk which up until now was considered unsuitable for the manufacturing of evaporated milk.

REFERENCES

ROY, N. K. and YADAV, P. L. 1972. A process for manufacture of evaporated milk. Patent (India) No. 132/65.

SINDHU, J. S. and TAYAL, M. 1984. Influence of sodium citrate on the heat stability of buffalo milk and its concentrate. Food Chem. *15,* 57-62.

SNEDCOR, G.W. and COCHRAN, W. G. 1968. *Statistical methods.* 6th Ed. Oxford and IBH Publishing Company, Calcutta, India.

SRINIVASAN, M. R., BHANUMURTHI, J. L. and SAMLIK, O. 1967. The present progress and problem encountered in the manufacture of (i) Evaporated or sweetened condensed milk (ii) Milk based baby foods (iii) Milk Powder. Indian Dairyman *19,* 173-175.

SWEETSUR, A. W. M. and MUIR, D.D. 1980. The use of permitted additives and heat treatments to optimise the heat stability of skim milk and concentrated skim milk. J. Soc. Dairy Tech. *33,* 101-105.

TAYAL, M. 1983. Heat stability and salt balance of buffalo milk and its concentrate (1:2) as influenced by homogenization and additives. M. Sc. Dissertation, Kurukshetra University, Kurukshetra.

TAYAL, M. and SINDHU, J. S. 1983. Heat stability and salt balance of buffalo milk as affected by concentration and addition of casein. J. Food Proc. Pres. *7,* 151-160.

VERMA, I. S. 1965. Study on the salt balance in pasteurized and cool aged milk with special reference to addition of calcium. Milchwissenschaft, *20,* 357-361.

PECTIC SUBSTANCES OF FABA BEANS AND THEIR RELATION TO TEXTURE OF COOKED BEANS

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ABSTRACT

Twenty samples of faba beans which showed wide variations in texture after cooking were analyzed for total pectin and pectin fractions (i.e., H_2O-, EDTA-, and NaOH-soluble fractions) contents of decoated seeds and seed coats. Total pectin ranged between 0.9 and 1.6% of the cotyledons and between 0.5 to 1.0% of the seed coats. Water-soluble pectin was the highest fraction with a mean value about 50% and 40% of the total pectin for the decoated seeds and the seed coats, respectively. No consistent significant correlation was found between the pectic substances fractions and the texture of cooked faba beans for both 1980 and 1981 samples except for the water-soluble pectin as a percentage of the total pectin content of decoated seeds ($r = 0.77$, $P < 0.01$ for penetrometer and $r = -0.76$, $P < 0.01$ for Kramer maximum shear force for 1980 and 1981, respectively).

INTRODUCTION

Mature dry seeds of *Vicia faba* L., like other legumes, vary widely in their cooking time. Mattson *et al.* (1950) suggested that softness of cooked peas depended upon the formation of water soluble pectin due to the exchange of divalent cations (Ca and Mg) with monovalent ones between pectic substances and phytates. Consequently, insoluble Ca-and Mg-phytates and soluble Na- and K-pectates (in the middle lamella) are formed causing easy separation between the cells and soft cooked peas.

Muller (1967), working on 13 varieties of peas and beans, come to the conclusion that besides the effect of free pectin, Ca, Mg and phytate contents, the cellulose and lignin contents of seed coats and cotyledon cell walls seem to be important in determining the softness of cooked pulses.

Kon (1968), working on beans that were stored for 4 years, found no correlation between cooking time and the concentration of total pectic

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substances or the concentration of any of the pectic fractions (H_2O-, EDTA-, and NaOH-soluble fractions).

This study is on the distribution of pectic substances between the seed coats and decoated faba bean seeds and their relation to softness of the pressure-cooked faba beans.

MATERIALS AND METHODS

Ten samples (20 kg each) of faba beans which varied widely in texture after cooking, were chosen from each of the 1980 and 1981 crops as described in a previous paper (Shehata *et al.* 1983). The texture of cooked beans was evaluated, as described later, before the samples were stored in a freezer (-20°C) until used for chemical analysis.

Chemical Determinations

Each sample (about 300 g) of faba beans was decoated manually using a sharp scalpel. Seed coats and decoated seeds were ground separately in an analytical mill (A 10-Janke & Kunkel, Ika-Werk Staufen, W. Germany) to pass a 60 mesh screen. Moisture content was determined by drying at 105°C for about 4 h. until a constant weight was reached (AOAC 1975). Pectic substances were fractionated using distilled water, 0.4% EDTA and 1 N sodium hydroxide solutions. Extraction was carried out after enzymatic hydrolysis of starch, then colorimeteric determinations were carried out according to the method of Dietz and Rouse (1952). All determinations were carried out in triplicate. Means of the standard errors were ± 0.010, ± 0.014, ± 0.006 for water-soluble, EDTA-soluble and NaOH-soluble pectin fractions, respectively of decoated seeds and were ± 0.002, ± 0.003 and ± 0.005 for the same fractions of seed coats.

Dry faba beans were cooked by autoclaving (seeds: distilled water; 1:4 W/V) at 115.5°C for 2h. The cooked beans were drained through cheese cloth and left to cool to room temperature (25°C) before texture evaluation.

Texture Evaluation

Mean softness score was reported as the number of soft beans in a random sample of ten cooked beans evaluated by ten experienced judges who were chosen by duo-trio test (Kramer and Twigg 1962). One hundred beans of each sample were tested individually by a Universal Precision penetrometer (Hartman 1976) using a 100-g weight and a penetration needle (Fisher Sci. Co., 1979-Cat. No., 13-401-10). The mean value of penetration

(in 0.1 mm) was presented as penetrometer reading. The higher the penetrometer reading, the softer the bean sample. Since the determination of the penetrometer readings is tedious and time consuming, the texture of 1981 crop samples was measured using the O.T.M.S. (i.e., Ottawa Texture Measuring System, Canners Machinery Ltd., Ontario, Canada) which was acquired in 1981. This system includes a 9005 Mainframe Daytronic Digital Indicator (Model SP-G5P, Riken Denshi Co., Ltd., Japan) for recording force deformation curves. Drained and cooled 100-g samples of cooked beans were placed in a standard Kramer shear-compression cell (Cat. No. CS-1) and the maximum (peak) force during the deformation was electronically detected and expressed as Kramer maximum shear force (Kg force/100g sample). This was carried out in triplicate. The smaller the shear force the softer the bean sample.

Simple linear correlation coefficients between the pectic substances and the texture evaluations (softness score, penetrometer reading and Kramer maximum shear force) were calculated according to Snedecor (1962).

RESULTS AND DISCUSSION

Results of texture measurements of cooked faba beans (Table 1) showed wide variations among the samples; standard deviations and coefficients of variability were quite high. The texture of bean samples showed more or less the same trend whether determined subjectively by taste panel (softness score) or objectively by penetrometer or Ottawa Texture Measuring System. Significant correlations were found between the softness score and the penetrometer reading ($r = 0.83$, $P < 0.01$) and between softness score and Kramer maximum shear force ($r = -0.77$, $P < 0.01$). The negative sign is due to the inverse relationship between Kramer shear force and softness.

Data about the total pectin and the pectic substances fractions in the decoated seeds and seed coats are shown in Tables 2 and 3. The total pectin content of decoated seeds varied from about 0.9 to 1.6 over the two years. White (1966) reported 1.69% total pectin for decoated faba bean seeds; but higher values (1.5 to 2.8%) were recorded for *Phaseolus vulgaris* whole seeds depending on the method of extraction (Kon 1968). The total pectin content of seed coats ranged between 0.6% to 1.0% for 1980 samples, but was almost constant at about 0.5% for 1981 samples. These data indicate that the pectin concentration in the seed coats was about 50 to 60% of that in decoated seeds.

Variations in pectic substances concentration occurred within the samples of the same variety. Moreover, the means of pectic substances fractions varied from one year to another. The water soluble pectin

Table 1. Texture of cooked faba bean samples

Sample[a] No.	1980 Crop		1981 Crop	
	Penetrometer Reading	Softness Score	Kramer Maximum Shear Force (Kg/100g)	Softness Score
1	36.7	5.8	115.4	6.7
2	35.6	6.4	134.6	7.2
3	17.8	4.7	157.6	4.1
4	17.6	3.6	117.4	2.0
5	46.8	9.5	122.6	5.4
6	65.9	9.1	52.7	8.6
7	23.0	7.5	54.6	8.9
8	70.3	8.4	56.9	9.4
9	68.3	9.2	64.9	8.8
10	69.7	8.8	61.9	8.0
Range	17.6–70.3	3.6–9.5	52.7–157.6	2.0–9.4
Mean	45.2	7.3	93.8	6.9
Standard deviation	22.0	2.1	39.5	2.4
Coefficient of variability	48.7	28.1	42.1	34.7

[a] Cultivars as presented in Tables 2 and 3 for 1980 and 1981 crops

concentration was the highest as compared with the other two fractions (i.e., EDTA-, and NaOH-soluble). Kon (1968) reported the highest concentration for the 0.05N NaOH-soluble fraction followed by the 1% EDTA-soluble and H_2O- soluble fractions in *Phaseolus vulgaris.*

The effects of pectic fractions on the texture of cooked beans should be dependent more on relative than on absolute concentrations. Therefore, the pectic fractions as percentages of the total pectin were calculated and are presented in Tables 4 and 5. Wide variations can be seen within each variety and between different years. The mean values for the H_2O-soluble fractions were about 50 and 40% of the total pectin for decoated seeds and seed coats, respectively, for both years. Mean EDTA-soluble pectin was much higher as a percentage of the total in 1980 samples than in 1981 samples. Means for the NaOH-soluble fractions were more or less the same (35%) for seed coats for the two year samples, but that for decoated seeds was much lower in the 1980 than in the 1981 samples.

Table 2. Pectic substances content of faba bean seeds (1980 crop; % dry basis)

Sample No.	Cultivar	Total Pectin		Pectic Substances					
				Water-Soluble		EDTA-Soluble		NaOH-Soluble	
		Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat
1	Giza 1	1.50	0.86	0.71	0.33	0.58	0.21	0.21	0.32
2		1.39	0.83	0.60	0.32	0.59	0.22	0.21	0.29
3		1.41	0.84	0.65	0.34	0.56	0.21	0.20	0.28
4		1.60	1.01	0.77	0.38	0.48	0.35	0.35	0.28
5		1.09	0.71	0.56	0.24	0.39	0.23	0.13	0.24
6		1.11	0.73	0.59	0.24	0.38	0.22	0.14	0.27
7		1.57	0.86	0.64	0.32	0.48	0.24	0.13	0.38
8	Giza 2	1.13	0.80	0.65	0.32	0.36	0.21	0.12	0.28
9		1.31	0.83	0.71	0.33	0.48	0.24	0.12	0.27
10		1.17	0.66	0.61	0.24	0.39	0.21	0.12	0.21
Range		1.09-1.60	0.66-1.01	0.56-0.77	0.24-0.38	0.36-0.59	0.21-0.35	0.12-0.35	0.21-0.32
Mean		1.32	0.81	0.65	0.30	0.47	0.235	0.17	0.27
Standard deviation		0.199	0.097	0.065	0.049	0.086	0.041	0.071	0.028
Coefficient of variability		15.0	11.9	10.0	16.1	18.4	17.5	41.2	10.4

Table 3. Pectic substances content of faba bean seeds (1981 crop; % dry basis)

Sample No.	Cultivar	Total Pectin		Pectic Substances					
				Water-Soluble		EDTA-Soluble		NaOH-Soluble	
		Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat
1	Giza 1	1.14	0.51	0.65	0.20	0.27	0.14	0.24	0.18
2		1.06	0.56	0.70	0.22	0.19	0.16	0.19	0.18
3		1.05	0.54	0.60	0.24	0.20	0.17	0.22	0.14
4		0.99	0.55	0.51	0.21	0.20	0.16	0.26	0.18
5		1.20	0.53	0.68	0.24	0.40	0.15	0.12	0.14
6	Giza 2	1.26	0.52	0.63	0.21	0.29	0.11	0.34	0.19
7		1.07	0.56	0.33	0.19	0.45	0.10	0.28	0.28
8		0.91	0.57	0.38	0.20	0.22	0.09	0.31	0.28
9		1.06	0.51	0.53	0.22	0.28	0.12	0.26	0.18
10		1.05	0.55	0.46	0.25	0.27	0.10	0.32	0.19
Range		0.91-1.26	0.51-0.57	0.33-0.70	0.19-0.25	0.19-0.40	0.09-0.17	0.12-0.34	0.14-0.28
Mean		1.08	0.54	0.54	0.22	0.28	0.13	0.25	0.19
Standard deviation		0.10	0.02	0.12	0.02	0.09	0.03	0.06	0.05
Coefficient of variability		9.30	4.00	22.7	9.10	31.4	22.6	25.9	25.1

Table 4. Pectic fractions as percentages of total pectin in faba bean seeds (1980 crop)

Sample[a] No.	Pectin Fractions					
	Water-Soluble		EDTA-Soluble		NaOH-Soluble	
	Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat
1	47.7	38.2	38.4	25.0	13.8	36.7
2	42.9	37.9	43.1	26.7	14.9	35.3
3	46.2	41.2	39.8	25.6	14.0	33.2
4	48.2	37.5	30.2	34.4	21.6	28.1
5	51.8	33.1	36.0	32.5	12.2	34.3
6	52.8	35.5	34.5	30.0	12.7	36.4
7	51.1	37.2	38.5	28.4	10.3	43.3
8	57.6	39.4	31.8	25.8	10.5	34.7
9	54.2	39.6	36.7	28.4	9.1	32.0
10	54.6	35.4	34.4	32.3	11.0	32.3
Range	42.9-57.6	33.1-41.2	30.2-43.1	25.0-34.4	9.1-21.6	28.1-43.3
Mean	50.7	37.5	36.3	28.9	13.0	34.6
Standard deviation	4.4	2.3	3.8	3.3	3.5	3.9
Coefficient of variability	8.7	6.3	10.6	11.3	27.1	11.4

[a] Cultivars as in Tables 2.

Table 5. Pectic fractions as percentages of total pectin in faba bean seeds (1981 crop)

Sample No. [a]	Pectin Fractions					
	Water-Soluble		EDTA-Soluble		NaOH-Soluble	
	Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat	Decoated Seeds	Seed Coat
1	49.4	38.0	23.4	27.1	20.9	34.9
2	65.6	38.5	18.1	28.9	18.1	32.5
3	57.4	44.0	19.0	30.7	20.9	25.3
4	51.4	38.8	19.9	28.6	26.6	32.9
5	56.7	45.0	33.6	27.9	9.6	27.1
6	49.9	41.0	23.4	22.0	26.7	36.9
7	31.1	33.1	42.4	17.4	26.5	49.6
8	41.3	34.9	24.1	16.4	34.5	48.7
9	50.1	42.3	25.8	24.0	22.8	34.8
10	44.2	46.0	25.3	18.5	30.5	35.4
Range	31.1-65.6	33.1-46.0	18.1-42.4	16.4-28.9	9.6-34.5	27.1-49.6
Mean	49.7	40.2	25.5	24.0	23.8	35.8
Standard deviation	9.5	4.2	7.4	5.3	6.9	7.9
Coefficient of variability	19.0	10.6	28.8	22.1	29.7	22.0

[a] Cultivars as in Tables 3.

Although significant correlations were found between certain pectic fractions of decoated seeds and texture of cooked beans of 1980 samples ($r = -0.69$, $P < 0.05$ for the EDTA-soluble fraction and $r = -0.88$, $P < 0.01$ for NaOH-soluble fraction with softness score and $r = -0.68$, $P < 0.05$ for penetrometer reading), no corresponding relations were repeatable for 1981 samples. However, the water-soluble fraction as a percentage of total pectin of the decoated seeds was significantly correlated with the texture of cooked beans for both years ($r = 0.77$, $P < 0.01$ for penetrometer reading of 1980 samples, $r = -0.76$, $P<0.01$ for Kramer maximum shear force for 1981 samples). These results indicate that the water-soluble pectin fraction as a percentage of the total pectin of the dry decoated seeds is more important than absolute pectin fraction contents in affecting the texture of cooked beans. Kon (1968) found no significant differences in the pectic fractions, as percentage of total pectin, from fast-cooking and slow cooking beans.

Jones and Boulter (1983) explained that the decrease of cooking rate in *Phaseolus* during storage was due to formation of insoluble pectin. Solubility of pectic fractions present in the dry beans may be influenced during cooking by the exchange between monovalent and divalent cations present in the beans.

ACKNOWLEDGMENTS

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REFERENCES

A.O.A.C. 1975. *Official Methods of Analysis,* A.O.A.C., 12th Ed. Association of Official Analytical Chemists, Washington, D.C.

DIETZ, J. H. and ROUSE, A. R. 1953. A rapid method for estimating pectin substances in citrus juices. Food Res. *18,* 169-177.

HARTMAN, G. H. 1976. Evaluating cultured product quality with the penetrometer. Cultured Dairy Product. J. *11,* 20-22, 28.

JONES, P.M.B. and BOULTER, D. 1983. The cause of reduced cooking rate in *Phaseolus vulgaris* following adverse storage conditions. J. Food Sci. *48,* 623-624 & 649.

KON, S. 1968. Pectic substances of dry beans and their possible correlation with cooking time. J. Food Sci. *33,* 437-438.

KRAMER, A. and TWIGG, B. A. 1962. *Fundamentals of Quality Control for the Food Industry,* AVI Publishing Co., Westport, CT.

MATTSON, S., AKERBERG, E., ERIKSSON, E., KOUTLER-ANDERSSON, E. and VAHTRAS, J. 1950. Factors determining the composition and cookability of peas. pp 40-61. Ann. Rev. Agric. Coll., Sweden.

MULLER, F. M. 1967. Cooking quality of pulses. J. Sci. Fd. Agric. *18,* 292-295.

SHEHATA, A. M. EL-TABEY, ABOU-BAKR, T. M. and EL-SHIMI, N. M. 1983. Phytate, phosphorus and calcium contents of mature seeds of *Vicia faba* L. and their relation to texture of pressure-cooked faba beans. J. Food Proc. Pres. *7,* 185-192.

SNEDECOR, D. W. 1962. *Statistical Methods.* The Iowa State Univ. Press, Ames, Iowa.

WHITE, J. L. 1966. The composition of the broad bean seed *(Vicia faba).* J. Exptl. Bot. *17,* 195-203.

MICROBIAL STABILIZATION OF INTERMEDIATE MOISTURE FOOD SURFACES I. CONTROL OF SURFACE PRESERVATIVE CONCENTRATION

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ABSTRACT

Changing environmental conditions to which intermediate moisture foods (IMF's) are exposed during production, storage, distribution and use, are important microbial stability factors. Temperature changes result in local surface condensations leading to microbial outgrowth on the surface. An approach to improved surface stability using a high preservative surface concentration maintained by an impermeable edible food coating was developed. Permeability tests predicted that zein was an acceptable coating. Sorbic acid distribution experiments confirmed its barrier properties. Apparent diffusion coefficient was estimated between 3 and 7 $\times$ 10^{-9} cm^2/s. These values were 150-300 times smaller than the value measured in the bulk of the IMF model food system used in this study, 10^{-6} cm^2/s.

INTRODUCTION

Developing an intermediate moisture food (IMF) can be conceptualized by representing shelf-life and organoleptic quality as a function of the fabrication parameters. An example of this approach, corresponding to the work done in our laboratories to prepare an intermediate moisture cheese analog, whose development and stability was previously reported

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(Motoki *et al.* 1982), illustrates the product development difficulties surrounding IMF technology (Fig. 1). The search for a region of potentially acceptable product formulations was guided by three controlling parameters: (1) an acceptable texture, mechanical measurements with an Instron; (2) an acceptable taste, sensory analysis tests; and, (3) a minimum water activity (a_w), measurements with an electric hygrometer. The area of acceptable combinations, represented by the shaded area, is quite limited. This points out that it is not always feasible to include considerations on product abuse situations. Storage temperature fluctuations (Grundke and Kuklov 1980), product transfers between facilities at different temperatures, or packaging products while still warm affect microbial stability of food surfaces (Torres 1984; Torres *et al.* 1985).

Another source of surface microbial stability problems is product handling. Even though some products are cooked or pasteurized, slicing and packaging gives ample opportunity for surface recontamination (Stiles and Ng 1979). A problem associated with surface contamination is that viable counts can be highly variable (Anderson *et al.* 1980; Gill 1979). This is particularly important in IMF's since bacteriostatic barriers can be overcome by a large number of cells. Furthermore for some microorganisms, in particular for *Staphylococcus aureus,* the minimal a_w requirement depends on oxygen concentration. Under anaerobic conditions the

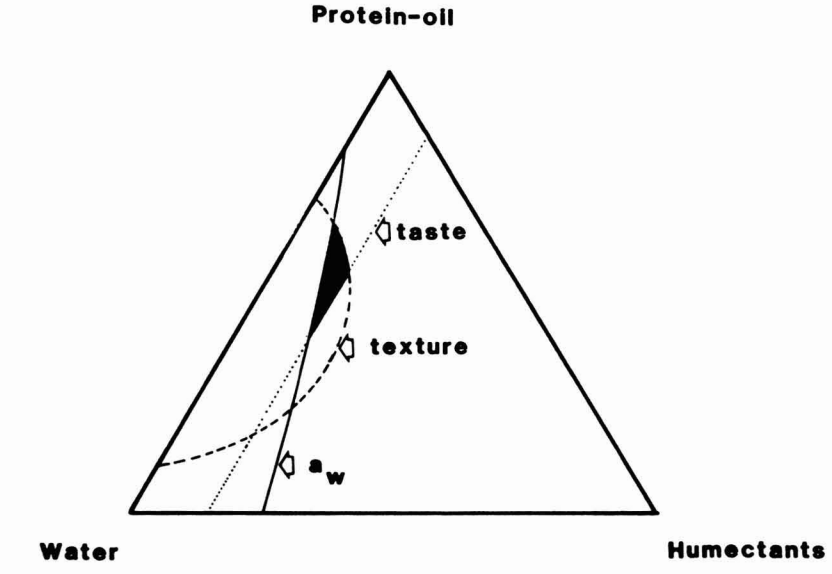

FIG. 1. IDENTIFICATION OF POTENTIAL FORMULATIONS FOR AN INTERMEDIATE MOISTURE CHEESE ANALOG Shaded area represents the region of potentially acceptable formulations. Product development details reported by Motoki *et al.* (1982)

minimal a_w for *S. aureus* is 0.91 while under aerobic conditions it is 0.86 (Scott 1953). Therefore, the surface of foods, where oxygen could be more readily available is the region we should be more concerned with potential outgrowth of this ubiquitous organism.

Therefore, there is a need to develop treatments that enhance surface resistance to microbial outgrowth. This paper shows that surface microbial stability can be improved by maintaining an unequal preservative distribution, i.e., start with a higher (initial) concentration of preservative(s) on the surface and use a coating to maintain this concentration difference for as long as possible. This approach requires the selection of a coating capable of reducing preservative diffusion from food surface into food bulk (Fig. 2). The model system used was the IMF soy-based cheese already cited (Motoki *et al.* 1982).

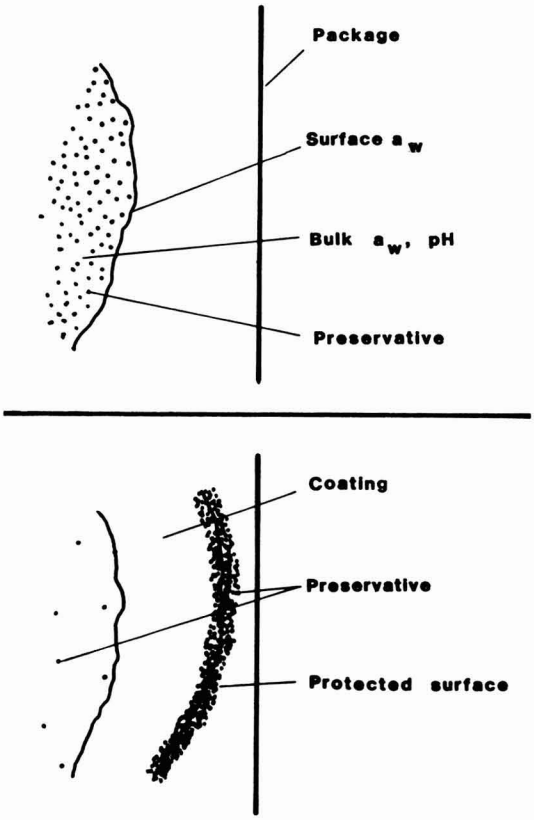

FIG. 2. SCHEMATIC REPRESENTATION OF MICROBIAL STABILITY ENHANCEMENT BY A HIGH CONCENTRATION OF K-SORBATE DEPOSITED ON A HIGHLY IMPERMEABLE FOOD COATING a. uncoated food with the recommended (Anonymous 1978) K-sorbate concentration use level evenly distributed b. coated food with a high surface K-sorbate concentration

MODEL DEVELOPMENT

Mass Transfer Estimations

Order of magnitude estimates were made of what rate would be required to achieve significant microbial stability improvements. Approximations for required diffusion values (D) can be based on the following unidimensional diffusion equation (Crank 1975, Eq. 2-7):

$$C(x) = \frac{M}{(\pi Dt)^{1/2}} \exp\left(-\frac{x^2}{4Dt}\right) \quad (1)$$

M = K-sorbate amount deposited on the surface, g/cm^2.

For small x values, i.e., surface conditions, this expression can be simplified to:

$$C(x) = \frac{M}{(\pi Dt)^{1/2}} \left(1 - \frac{x^2}{4Dt}\right) \quad (2)$$

It is difficult to define accurately 'surface' to determine what 'reduction in surface concentration' will result in microbial stabilization loss. For estimation purposes an 'average surface concentration' was defined as follows:

$\overline{C}(O, \Delta x)$ = average between x = 0 and x = Δx

$$\cong \frac{M}{(\pi Dt)^{1/2}} \quad (3)$$

The average value at time 0 can be defined as:

$$\overline{C_0}(0, \Delta x) = M/\Delta x \quad (4)$$

Let us define reduction in average surface concentration as:

$f = \overline{C}(0, \Delta x)/\overline{C_0}(0, \Delta x)$

$$= \frac{\Delta x}{(\pi Dt)^{1/2}} \quad (5)$$

This equation can be simplified to yield:

$$D \cong \frac{\Delta x^2}{f^2 \pi t} \quad (6)$$

Assuming that x is the coating thickness h we can estimate required D-values for a given desired stability period and assumed f value (= 0.05,

Table 1. Apparent coating diffusion coefficients for sorbic acid required to achieve significant microbial stability improvements

Days	D, cm^2/sec		
	h = 0.01 mm	h = 0.03 mm	h = 0.05 mm
	$\times 10^{-10}$	$\times 10^{-9}$	$\times 10^{-9}$
1	14.7	13.3	36.8
5	2.9	2.7	7.4
10	1.5	1.3	3.7
30	0.5	0.4	1.2

Table 1). These values are compared with the one obtained by Guilbert *et al.* (1983). Their value, determined in an IM agar model was 2×10^{-6} cm^2/s, i.e., to be effective, a coating will have to allow K-sorbate diffusion about 1,000 times slower than IM food-like matrices.

Coating Film Permeability Measurements

The permeability cell described in Fig. 3 was used to determine the permeability constants of coating films supported by regenerated cellulose. Under well stirred conditions, the resistance to mass transfer for an effective coating (low D value) can be assumed to be due only to film properties. A constant concentration difference between reservoirs facilitates the analysis of experimental data. This was done by using:

c_1 (t = 0) = concentration in reservoir 1 = 0
c_2 (t = 0) = concentration in reservoir 2 = large
= 10 to 100 (mg K-sorbate/ml solution)

The use of large reservoirs compared to the area of mass transfer resulted

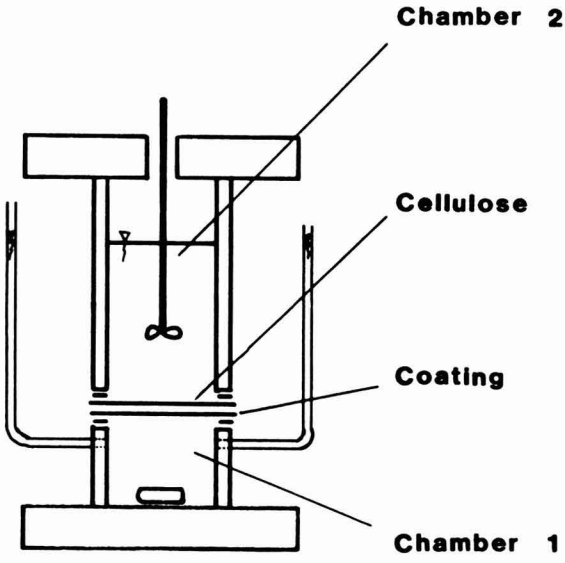

FIG. 3. PERMEABILITY CELL

in constant permeability rates equal to:

$$N = DA\,(c_2 - c_1)/x \tag{7}$$

where:

N = flux of K-sorbate
D = apparent diffusion constant
A = area of mass transfer
c_i = concentration in the film which is in equilbrium with bulk solution concentration c_i; equilibrium expressed as $c_i = kc_i$
x = a diffusion distance

Assuming constant k we obtain:

$$N = KA(c_2 - c_1) \tag{8}$$

where:

$$K = kD/x \tag{9}$$

The use of regenerated cellulose, as a reference permeability value, provided an opportunity to obtain a rapid, even though approximate

measurement, of coating effectiveness. Assuming no interface resistance and k values independent of testing material the following expressions are valid (r = cellulose, rc = coated cellulose and c = coating film):

$$K = k_i D_i / x_i \text{ with } i = r, rc, c \tag{10}$$

$$K_r = k_{rc} = k_c \tag{11}$$

$$\frac{x_{rc}}{D_{rc}} = \frac{x_r}{D_r} + \frac{x_c}{D_c} \tag{12}$$

where:

$$x_{rc} = x_r + x_c \tag{13}$$

We can express x_c as:

$$x_c = f' x_r \tag{14}$$

Thus:

$$\frac{1 + f'}{D_{rc}} = \frac{f' D_r + D_c}{D_r D_c} \tag{15}$$

We are interested in $D_c \langle\langle f D_r$, thus:

$$1 + f' = f D_{rc}/D_c \tag{16}$$

From (10, 13 and 14) we obtain:

$$\frac{k_{rc}}{k_r} = \frac{D_{rc} x_r}{D_r x_{rc}} = \frac{D_{rc}}{(1 + f')D_r} \tag{17}$$

Finally from (14, 16 and 17) we obtain:

$$\frac{D_c}{D_r} = \frac{x_c K_{rc}}{x_r K_r} \tag{18}$$

This simple expression allowed us to use permeability values to predict whether a coating film had the desired effect on sorbic acid diffusion rate.

MATERIALS AND METHODS

Permeability Experiments

As shown in Fig. 3 the permeability cell was provided with mechanical and magnetic stirrers to reduce resistance at the interfaces. The side tubes were used to load the cell with the high concentration solution, eliminate

air bubbles and adjust levels to eliminate the influence of hydrostatic pressure on permeability. Experiments were run at room temperature. Samples, < 1 ml, were taken from the upper chamber, diluted in glycerol solution, and preservative concentration determined spectrophotometrically (at 268 nm, absorption maximum determined experimentally) with no need for an extraction procedure. A 50% glycerol (w/w) solution was used to have a a_w level in the intermediate moisture range. This was important since we wanted a coating film sorption status similar to its actual use conditions.

The advantages of regenerated cellulose (dialysis membrane type 30F0, thickness = 30 microns, Union Carbide, Chicago, Illinois) were its availability, inertness, mechanical strength and low cost. Dialysis tubing made out of regenerated cellulose are very homogeneous, thus facilitating the obtention of reproducible results.

IMF Model Studies

IMF Model Samples Preparation

The IMF chosen for testing was a cheese analog developed previously (Motoki *et al.* 1982), with the composition given in Table 2. Glycerol (Certified A.C.S., Fisher Scientific Co, Fair Lawn, NJ), sodium chloride (Certified A.C.S., Fisher Scientific Co.) and sorbitol (Pfizer Co., New York, NY) were dissolved in warm water (about 50C) with sorbic acid (Pfizer Co.) as the mycostatic agent. An emulsion paste was obtained by preblending 30% of the proteins in a Waring Blendor with the aqueous solution. The remainder of the protein was mixed with an oil phase composed of hydrogenated vegetable oil (Durkex 500, SCM Corp., New York, NY) and decaglycerol decaoleate (Glyco Chemical Inc., Greenwich, CT) and then emulsified using a food cutter (2 blades, 1450 rpm, Kitazawa Sangyo, Co., Tokyo, Japan). This emulsion was then blended with the emulsion paste using the same food cutter. Some of the water was then evaporated by blowing warm air into the food cutter bowl. When the desired a_w level was reached, 0.88, the emulsion was filled into a cellulose casing (Type 30F0, Union Carbide, Tarrytown, NY) with a hand press. Thereafter, casings were placed into seamless vinylidene chloride casing tubes (diameter 40 mm, Kreha Chemical Co., Tokyo, Japan).

The emulsion was then heated for 2 h in a water bath held at 85C. After cooling, pH was determined with a surface electrode probe (combination electrode 39507, Beckman Instruments, Inc., Cedar Grove, NJ). Finally a_w was measured using an electric hygrometer (SINA Equihygroscope,

Table 2. IMF model composition

INGREDIENT	g
Isolated soy protein	26.1
Na-caseinate	5.9
Ca-caseinate	2.0
Hydrogenated vegetable oil	34.0
Decaglycerol monooleate	0.4
Salt	4.8
Glycerol	5.9
Sorbitol	19.3
K-sorbate	variable
Water[a]	--

[a] The initial water content is 100 ml/100g solids; this amount is reduced by drying the mixture with warm air so as to achieve the desired final a_w.

Nova Sina, Zurich, Switzerland; marketed in the USA by Beckman Instruments). Casings were then carefully removed under a laminar air flow hood. The cylinder thus obtained was cut into disks (r = 1.3cm, h = 1 cm) and placed on sterile dissecting needles. After 12 h storage they were hand sprayed for 20 s using a 125 ml glass atomizer (Fisher Scientific Co., Pittsburg, PA). The zein solution was prepared by dissolving 10 g zein (Colorcon, West Point, PA), 2.5 g glycerol (Certified A.C.S., Fisher Scientific Co.) and 1 g Myvacet (Kodak Chemical Reagents, Rochester, NY) and kept warm by using a 50°C water bath.

To maintain constant a_w, samples were kept in a constant 88% RH chamber (over saturated $BaCl_2$) before and after zein spraying. Air in the chamber was kept in circulation by the use of a fan. The chamber itself was placed in a constant temperature room (35°C).

Sorbic Acid Distribution Studies

To show that a coating reduced preservative diffusion from coated food surface into food bulk to an extent consistent with the measured steady state permeability cell experiments, coated and uncoated IM model samples were separated into a core and a surface fraction. From each disk shaped sample (r = 1.3 cm, h = 1 cm) we obtained a smaller disk (r = 0.8 cm, h = 0.5 cm); the rest was the surface fraction. Sorbic acid concentration in each fraction was then determined by HPLC, using a procedure based on the work by Park and Nelson (1981). The HPLC system consisted of: a high pressure pump (M-6000A, Waters Associates, Milford, MA); a variable wavelength detector (Model 450, Waters Associates); a high pressure loop injector (CV—6UHPa—N60, Valco Instruments, Co., Houston, TX); a signal integrator (Model 3390A, Hewlett Packard Corp., Avondale, PA) and a reverse phase HPLC column (μ Bondapak C-18, 39mm x 30cm, Waters Associates).

Samples were accurately weighed into a Waring Blendor jar and 5g Celite 545 (Supelco, Reagent grade) and 100 ml methanol (Omnisolv, MCB Reagents, Gibbstown, NJ) were added. After mixing at medium speed for 4 min, the slurry was filtered on a Buchner funnel through Whatman No. 2 paper, the jar and the solids were rinsed with 50 ml methanol and the filtrate was diluted to 250 ml with methanol. The extract was transferred to a separatory funnel and partitioned by the addition of 100 ml 0.5N NaOH and 50 ml of a 1:1 mixture of petroleum ether and ethyl ether mixture. The aqueous phase was transferred to a 1,000 ml Erlenmeyer. The organic layer was gently washed with 15 ml 0.5% N NaOH and then discarded. The aqueous extracts were combined, titrated to pH 2.0 with HCl (1 + 1) and the total volume accurately determined. A 5 ml aliquot was then passed through a Millipore filter (EHWP 01300, Milipore Corp., Bedford, MA) and 20 μl were injected onto the HPLC column with mobile phase flowing at 1.0 ml/min. Column effluent was monitored at 254 nm. Analysis of an IMF model prepared with 0.2% K-sorbate showed excellent recovery values, (0.199 $\pm$ 0.01)% K-sorbate, n = 8.

Microscopy Studies

Nomarsky microscopy (Peil 1982) was used to observe the general appearance of coated samples as well as to estimate coating thickness. Samples embedded in Paraplast (Sherwood Medical Industries, North Brunswick, New Jersey) and frozen in CO_2 were sliced into 10 microns sections and placed immediately on glass slides. To prevent artifacts

caused by dehydration, samples were kept in a constant humidity chamber (over saturated $BaCl_2$) and examined within 24 h.

RESULTS AND DISCUSSION

Permeability experiments

Uncoated cellulose sorbic acid permeability measurements, at an initial concentration differential of 10 mg sorbic acid/ml solution, gave an average value $K_r = (4.7 \pm 0.1)x10^{-2}$ (mg/h cm²)/(mg/ml), n = 5. Zein coated cellulose determinations, shown in Fig. 4 showed a concentration dependence. The effectiveness of zein films was evaluated by comparing experimental K-values, for coated and uncoated cellulose, obtained at the same initial concentration difference (10 mg sorbic acid/ml solution). The values were:

$K_r = 4.7 \times 10^{-2}$ (mg/h cm²)/(mg/ml)
$K_{rc} = 1.5 \times 10^{-4}$ (mg/h cm²)/(mg/ml) (from Fig. 4)
$x_r = 0.003$ cm (product specification)
$x_c = 0.00088 \pm 0.00012$ cm (SEM determination)

Substituting these values in Eq (19) we found zein films diffusion values for transport of sorbic acid 1,000 times lower than the cellulose film.

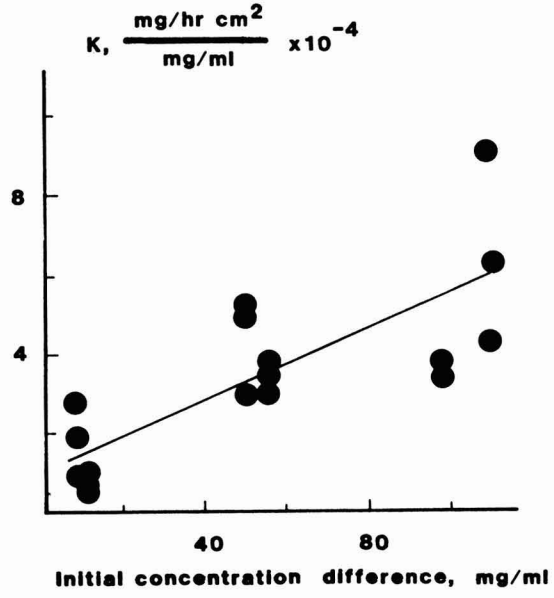

FIG. 4. VARIATION OF K-VALUES AS A FUNCTION OF INITIAL K-SORBATE CONCENTRATION DIFFERENCE

Microscope Studies

Photomicrographs in Fig. 5 show the IMF model with a continuous coating and minimal thickness variation. Thickness measurements, (n = 10) showed that each successive application resulted in similar thickness increments (12.0, 14.5, 11.5; average = 12.7 microns).

Sorbic Acid Determinations

Sorbic acid determinations showed variation in the amount of sorbic acid deposited on each individual sample as shown in Table 3. Controls indicate that this variation was not due to sample handling during fractionation into a surface and a core fraction. Data were normalized to solve this variation. Sorbic acid core concentrations were divided by the

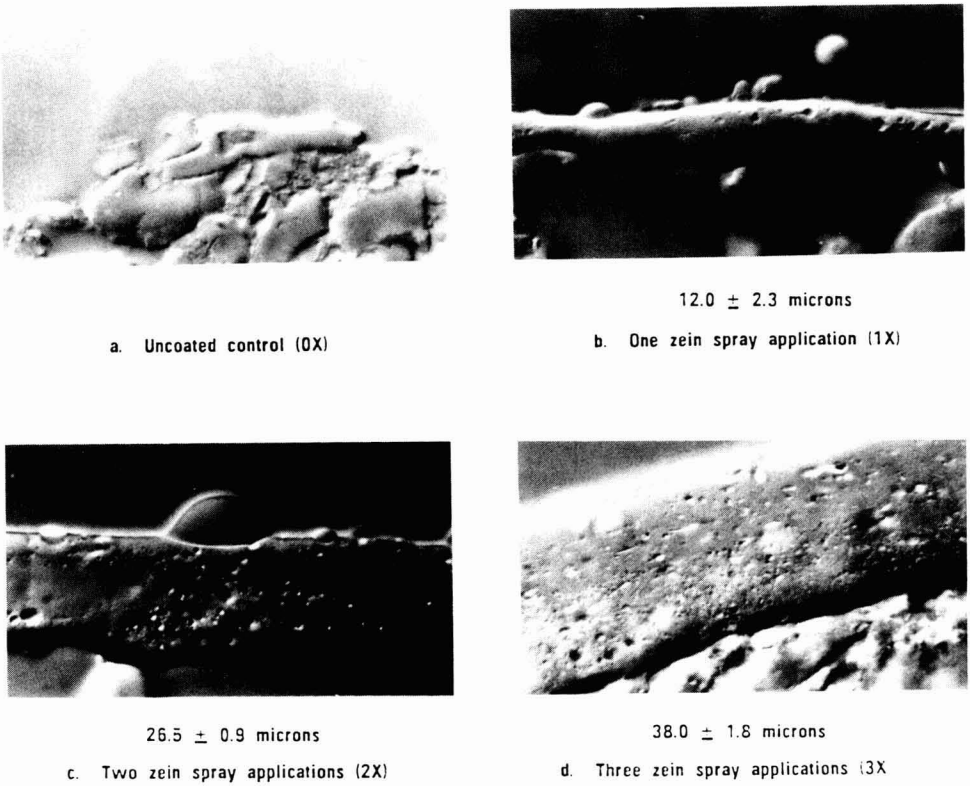

FIG. 5. NOMARSKY MICROSCOPY STUDIES a. uncoated control b. one zein spray application [1X], thickness = 12.0 ± 2.3 microns c. two zein spray applications [2X], thickness = 26.5 ± 0.9 microns d. three zein spray applications [3X], thickness = 38.0 ± 1.8 microns

Table 3. Total sorbic acid deposited on each individual IMF cheese sample

TIME, h	POOLED SAMPLES, mg [a,c]	RECOVERY CONTROLS, mg [b]
2	18.9 ± 6.8 (n = 5)	-
18	22.6 ± 5.3 (n = 6)	-
44	17.7 ± 2.1 (n = 6)	13.7
68	15.4 ± 6.1 (n = 6)	14.9; 16.4
118	20.0 ± 3.9 (n = 5)	12.9; 15.0
168	21.5 ± 10.2 (n = 5)	13.6
228	14.8 ± 4.0 (n = 6)	12.3; 9.1
	18.6 ± 6.1 (n = 39)	

[a] Corresponds to uncoated and coated samples
[b] Controls to determine if losses had occurred during fractionation
[c] n = number of samples

total amount deposited on each individual piece. Normalized values were then plotted as a function of sampling time and surface treatment. As shown in Fig. 6, zein coatings reduced sorbic acid core concentrations significantly. This reduction represents the diffusion rate reduction into the food of sorbic acid deposited on the surface due to the barrier properties of zein films. Moreover, the thickness, i.e., the number of zein spray applications had also a strong effect.

Apparent diffusion coefficients for sorbic acid in the food model and zein coating were evaluated using data from Fig. 6. The following assumptions were necessary: (1) Experimentally determined average core concentrations were assumed to represent the concentration in the center of the food piece. This is valid when the core sample is small. In our case it was 1/5 of the food piece, or about 1 g. (2) Solutions for unidimensional diffusion in an infinite slab were assumed valid for the analysis of our disk shaped samples (r = 1.3 cm, h = 1 cm) with diffusion occuring from every surface. This simplification was possible because edge effects were eliminated during sample fractionation, i.e. by obtaining a core piece shaped as a smaller disk (r = 0.8 cm, h = 0.5 cm).

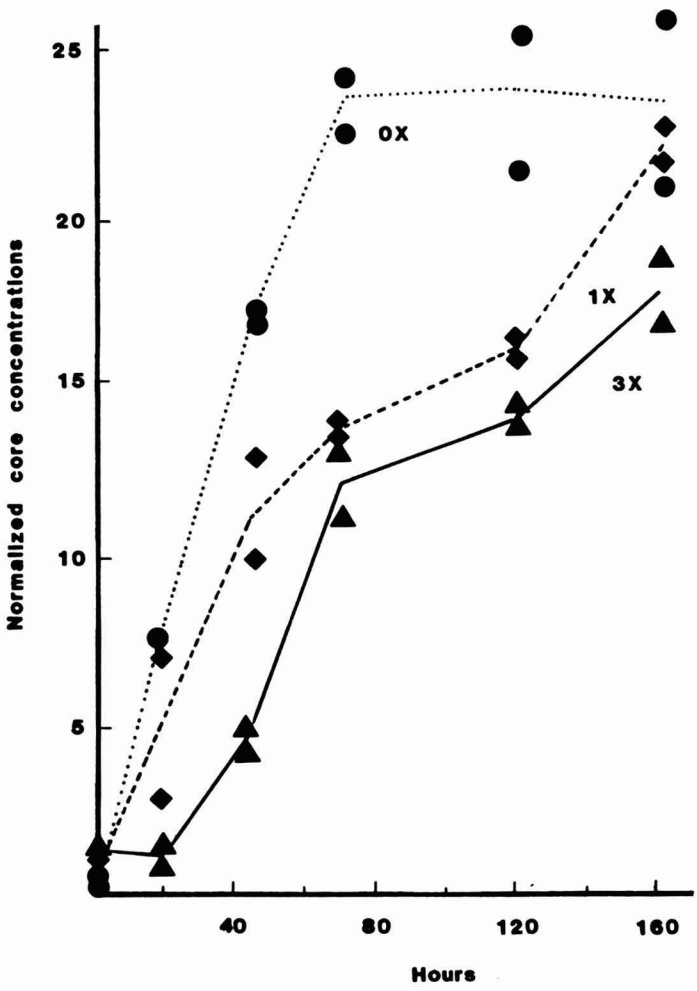

FIG. 6. SORBIC ACID DISTRIBUTION STUDIES Effect of coatings on normalized core concentrations defined as core concentration divided by total amount deposited on each individual piece. Experiment was run in duplicates, lines have been drawn through average values.

Center conditions can be evaluated using Gurney-Lurie graphs (Adams 1954, p. 36). Therefore, we defined:

a. Y = an unaccomplished core concentration change
$Y = (C - \overline{C})/\overline{C}$
C = core concentration (19)
$\overline{C}$ = average overall concentration

b. X = a relative time
$X = D_f t/r_f^2$
r_f = half thickness = 0.5 cm (20)
t = time, seconds
D_f = food apparent sorbic acid diffusion constant, cm^2/s

c. m = a resistance ratio
$m = (D_f r_f)/(D_c r_c)$ (21)
r_c = coating thickness = 0.0012 cm [for 1X samples)
= 0.0038 cm [for 3X samples)
D_c = coating apparent sorbic acid diffusion constant, cm^2/s

Determinations were done at various time intervals, with individual values summarized in Table 4. Calculated average values for the apparent diffusion coefficients for sorbic acid in the food model were ($1.0 \pm 0.1 \times 10^{-6}$, n = 6), and in the coating ($3.3 \pm 0.7 \times 10^{-9}$, n = 5) and ($6.8 \pm 0.9 \times 10^{-9}$, n = 6) cm^2/s for samples coated 1 and 3 times, respectively.

The value obtained for the uncoated IMF model, 1×10^{-6} cm^2/s, agrees well with the one obtained by Guilbert *et al.* (1983). Their value, determined in an IM agar model at the same a_w, 0.88, was $2.0 \times 10^{-6} cm^2/s$.

The calculated D_f/D_c ratios were 300 (1x) and 150 (3x). A ratio of 1,000 was obtained in the permeability cell experiments, comparisons based on cellulose film. The difference may be due to several factors. The surface of the IMF model is rich in topographical features such as pores, valleys, etc., which makes it more difficult to cover than cellulose. Second, the assumptions made to analyze Fig. 6, unidimensional diffusion and measured core concentration representing the situation in the piece center, introduce errors leading to lower D values. The values are however quite comparable given the orders of magnitude of diffusion reduction with respect to cellulose.

CONCLUSIONS

The use of high surface K-sorbate concentrations required the development of a coating acting as a diffusion barrier. A zein coating was identified through permeability cell experiments as an acceptable edible diffusion barrier. Sorbic acid distribution studies confirmed the barrier properties of zein films. The effectiveness of zein films has also been confirmed by extensive microbiological tests (Torres and Karel 1985). The performance of zein coatings in industrial practice remains to be determined, although some literature information is available (Mendoza 1975).

Table 4. Calculated apparent sorbic acid diffusion constant

a. D values calculated from uncoated samples, [0X]

time, h	Y	X	$D_f \times 10^6$, cm^2/sec
10	0.84	0.15	0.9
20	0.61	0.26	0.9
40	0.37	0.50	1.1
60	0.12	0.95	1.1
			1.0 ± 0.1

b. D values calculated from coated samples, [1X]

time, h	Y	X	M	$D_c \times 10^9$, cm^2/sec
60	0.46	0.85	0.55	4.4
80	0.39	1.14	0.70	3.4
100	0.35	1.42	0.75	3.2
120	0.31	1.71	1.00	2.4
140	0.18	1.99	0.75	3.2
				3.3 ± 0.7

c. D values calculated from coated samples, [3X]

time, h	Y	X	M	$D_c \times 10^9$, cm^2/sec
60	0.60	0.85	1.00	7.6
80	0.47	1.14	0.95	8.0
100	0.43	1.42	1.10	6.9
120	0.40	1.71	1.35	5.6
140	0.32	1.99	1.30	5.8
160	0.23	2.28	1.10	5.9
				6.8 ± 0.9

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REFERENCES

ADAMS, W. H. 1954. *Heat Transmission,* 3rd ed. McGraw-Hill Book Co, Inc., New York.

ANDERSON, M. E., SEBAUGH, J. L., MARSHALL, R. T. and STRINGER, W. C. 1980. A method for decreasing sampling variance in bacteriological analysis of meat surfaces. J. Food Protection *43,* 21.

ANONYMOUS 1978. Sorbic acid and potassium sorbate. Publication IC/FI-13, Monsanto Industrial Chemicals Co., St. Louis, MO.

CRANK, J. 1975. Methods of solution when the diffusion coefficient is constant. Chapter 2. In *The Mathematics of Diffusion,* 2nd Edition, p. 11. Oxford University Press, Ely House, London.

GILL, C. O. 1979. A review. Intrinsic bacteria in meat. J. Appl. Bacteriology *47,* 367.

GRUNDKE, G. and KUKLOV, K. 1980. Das Kryptoklima in Lebensmittel-verpackungen. Lebensmittelindustrie *27* (1), 13.

GUILBERT, S., GIANNAKOPOULOS, A. and CHEFTEL, J. C. 1983. Diffusivity of sorbic acid in food gels at high and intermediate water activities. Paper presented at the International Symposium on the Properties of Water, Beaume, France, Sept. 11-16.

MENDOZA, M. A. 1975. Preparation and physical properties of zein based films. Sc.M. Thesis, Univ. Mass., Amherst.

MOTOKI, M., TORRES, J. A. and KAREL, M. 1982. Development and stability of intermediate moisture cheese analogs from isolated soybean proteins. J. Food Proc. Pres. *6*(1), 41.

PARK, G. L. and NELSON, D. B. 1981. HPLC analysis of sorbic acid in citrus fruit. J. Food Science *46*(5), 1629.

PEIL, A. 1982. Development and characterization of fortified polymer coatings for rice. PhD. Thesis, M.I.T., Cambridge.

SCOTT, W. J. 1953. Water relations of *Staphylococcus aureus* at 30C. Austr. J. Biol. Sci. *6,*549.

STILES, M. E. and NG, L. K. 1979. Fate of pathogens inoculated onto vacuum-packaged sliced hams to simulate contamination during packaging. J. Food Protection *42,*464.

TORRES, J. A. 1984. Exploration of the stability of intermediate moisture foods. Ph.D. Thesis, M.I.T., Cambridge.

TORRES, J. A., BOUZAS, J. O. and KAREL, M. 1985. Microbial stabilization of intermediate moisture food surfaces. II. Control of surface pH. J. Food Proc. Pres. *9,* 00

TORRES, J. A. and KAREL, M. 1985. Microbial stabilization of intermediate moisture food surfaces. III. Effects of surface preservative concentration and surface pH control on microbial stability of an intermediate moisture cheese analog. J. Food Proc. Pres. *9,* 00

MICROBIAL STABILIZATION OF INTERMEDIATE MOISTURE FOOD SURFACES II. CONTROL OF SURFACE pH

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ABSTRACT

This paper explores the possibility of reducing surface pH to solve intermediate moisture foods microbial stability problems associated with localized surface condensations caused by temperature fluctuations. Surface pH reduction increases surface availability of the most active form of sorbic acid and other lipophilic acids used as preservatives.

A negatively charged macromolecule was immobilized in the form of a food surface coating component while other molecules, particularly electrolytes, move freely. The effect on surface pH was described using a Donnan equilibrium model that predicted a permanent pH difference between surface and food bulk. The key parameters were electrolyte concentration and the concentration of charged groups on the macromolecule. An IMF model with low total electrolyte concentration was coated with a deionized mixture of λ-carrageenan and agarose. Measured pH differential was 0.3 to 0.5 pH units. Such a pH reduction resulted in a calculated 2.5 fold increase in the surface availability of the active form of sorbic acid as compared to food bulk conditions.

INTRODUCTION

Most intermediate moisture foods (IMF) are not affected by changing external environmental relative humidity conditions. These products are generally packaged in moisture proof materials. However, temperature fluctuations can result in extensive surface condensation problems (Torres 1984). As a consequence, surface microbial growth is then often observed. This possibility is not taken into account in tests conducted

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under conditions of constant temperature and humidity (e.g., Hsu *et al.* 1983; Bhatia and Muhadar 1982; Erickson 1982; Theron and Prior 1980; Hanseman *et al.* 1980; Flora *et al* 1979; Pavey 1972; Anonymous 1972).

Examples of unsteady state temperature situations abound in the production and commercialization of IMF. A typical situation is the temperature pattern inside a food warehouse. Grundke and Kuklov (1980) reported fluctuations that were especially severe during the winter season when energy was saved by turning the heating system on and off. A similar situation can be expected in a consumer's kitchen, where an air conditioning or heating system is turned on and off. The constant repetition of these temperature cycles can result in severe microbial stability problems due to the repetitive creation of surface conditions with high water activity (a_w). Another common situation is the transfer of products between environments with very different temperature, e.g., a heated warehouse to a railroad car, which in winter time could be 30°F colder. Condensations caused by evaporative cooling can raise local surface a_w above the design safety value (Torres 1984).

Improved stability of surfaces could be achieved by lowering surface pH. For lipophilic acids commonly used as preservatives, e.g., sorbic acid, surface pH reduction increases surface availability of the most effective form of the preservative (undissociated acid) (Eklund 1983; Motoki *et al.* 1982; Akedo *et al.* 1977; Freese *et al.* 1973).

Preliminary experimental work showed that food samples with a reduced surface pH were capable of resisting surface growth significantly longer than untreated controls. In these tests, a non permanent pH difference between food bulk and food surface was achieved by inclusion of low molecular weight acids in a zein based coating described by Torres *et al.* (1985). Lactic acid was used in the example shown in Fig. 1. Samples were surface inoculated with *Staphylococcus aureus* S-6 and then stored at 37°C and 87% RH. pH was measured with a surface pH electrode (combination electrode 39507, Beckman Instruments, Inc., Cedar Grove, NJ). These tests showed also that, as soon as the pH difference disappeared, rapid growth occurred. Diffusion was assumed to be the mechanism for pH equilibration.

MODEL DEVELOPMENT

Donnan Equilibrium Model

The Donnan model for semipermeable membranes (Hiemenz 1977) can be used to analyze the possibility of establishing a permanent pH difference and to identify the parameters which allow conditions for maximum

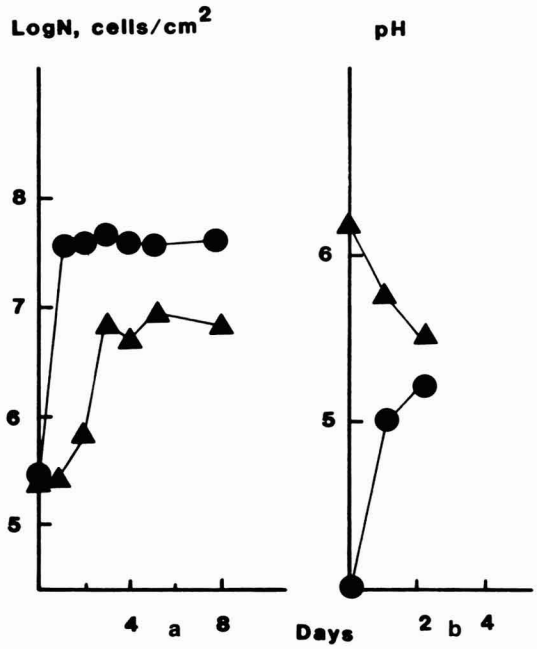

FIG. 1. PRELIMINARY EXPERIMENTAL WORK ON THE REDUCED SURFACE pH CONCEPT

a. *S. aureus* S-6 counts on coated and uncoated sample
▲ pH 4 zein coated samples
● uncoated control

b. pH values as function of location and time for zein coated samples.
● sample surface pH
▲ sample core pH

pH difference. It describes the concentration differentials between two solutions created by the presence of a membrane separating them which is permeable to low molecular wieght electrolytes but impermeable to a charged macromolecule (Grignon and Scallan 1980; Scallan and Grignon 1979; Donnan 1934, 1924, 1911; Donnan and Guggenheim 1932). It corresponds to the situation of a charged macromolecule (P^{-z}, z = number of charged groups), immobilized in the form of a food surface coating component, while other components, water and other solutes, particularly electrolytes (e.g., Na^+ and Cl^-), are able to move freely from the food bulk to the surface, and viceversa (Fig. 2). This situation can be further schematized as indicated in Fig. 3, where M-M' represents the theoretical membrane, M^+ and X^- are electrolytes and sides 1 and 2 correspond to the coating and food model, respectively. Using assumed molal concentration values n, m, y and x, and the equilibrium constant for water (K_w), the remaining expressions were derived from electroneutrality considerations.

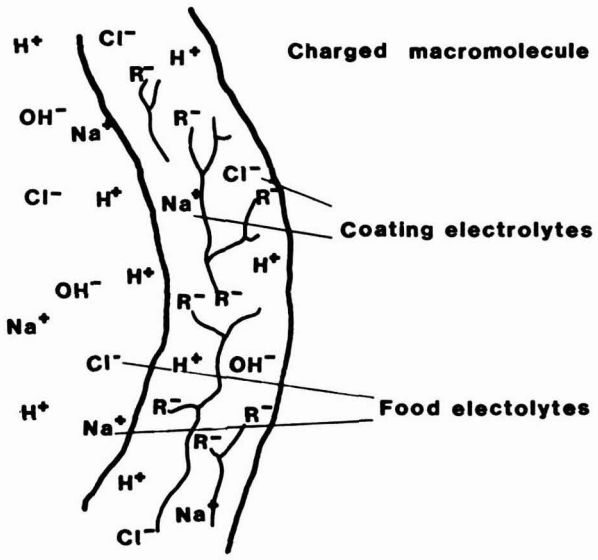

FIG. 2. IMMOBILIZATION OF A CHARGED MACROMOLECULE TO ESTABLISH A PERMANENT pH DIFFERENCE BETWEEN SURFACE AND FOOD BULK

COMPONENT	MOLAL CONCENTRATION		MOLAL CONCENTRATION
		M	
	SIDE 1		SIDE 2
M^+	$K_w/y + n + zmp-z - y$		$K_w/x + m - x$
X^-	n		m
H^+	y		x
OH^-	K_w/y		K_w/x
p^{-z}	---		
		M'	

FIG. 3. SCHEMATIC REPRESENTATION OF A SEMIPERMEABLE MEMBRANE

Identification of parameters for the prediction of the pH difference between surface and food bulk.

Derivation of the pH Differential Expression

The pH differential expression can be derived starting from the six particular cases of equilibrium deviations depicted in Fig. 4 (Hiemenz 1977). All mirror image deviations were not considered since they would yield identical equations. We should also remember that ($a_{j/i}$ = activity coefficients of component j; /i indicates side i, i = 1, 2; $a_{j/i} = 1$ for j = H_2O):

$$a_{OH^-/1}\, a_{H^+/1} = K_w \tag{1}$$

$$a_{OH^-/2}\, a_{H^+/2} = K_w \tag{2}$$

K_w = equilibrium constant for water

Applying the equilibrium condition to Case 1 (Hiemenz 1977):

$$dG = \mu_{X^-/1}\,(dn_{X^-/1}) + \mu_{OH^-/1}\,(dn_{OH^-/1}) + \mu_{X^-/2}\,(dn_{X^-/2}) + \mu_{OH^-/2}\,(dn_{OH^-/2}) = 0 \tag{3}$$

From mass and electroneutrality considerations:

$$dn_{X^-/1} = dn_{OH^-/1}\; dn_{X^-/2} = dn_{OH^-/2} = dn \tag{4}$$

Therefore:

$$\mu_{OH^-/1} + \mu_{X^-/2} = \mu_{OH^-/2} + \mu_{X^-/1} \tag{5}$$

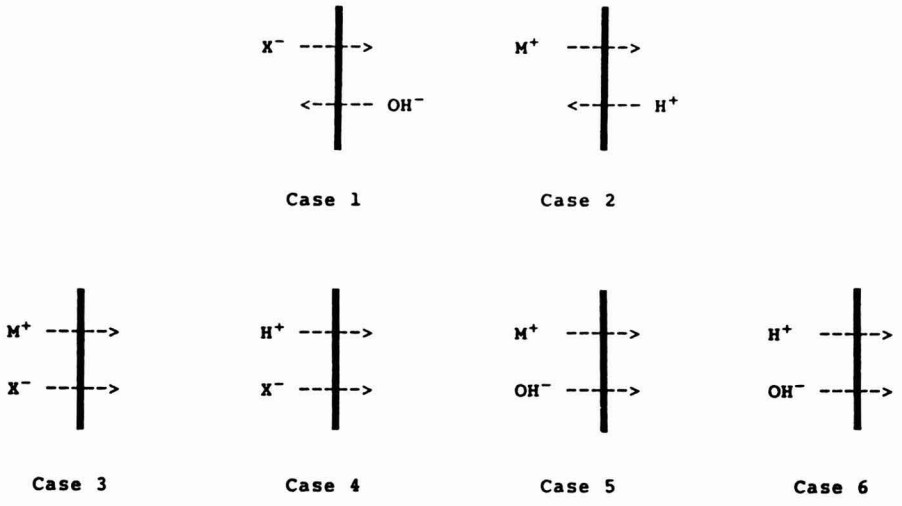

FIG. 4. EQUILIBRIUM DEVIATIONS USED FOR THE EQUILIBRIUM ANALYSIS OF A SEMIPERMEABLE MEMBRANE.

But:

$$\mu_i = \mu_i^0 + RT \ln a_i \tag{6}$$

Substituting in (5):

$$a_{X^-/2}\, a_{OH^-/1} = a_{X^-/1}\, a_{OH^-/2} \tag{7}$$

Similarly for cases 2 through 6 described in Fig. 4:

$$a_{M^+/2}\, a_{H^+/1} = a_{H^+/2}\, a_{M^+/1} \tag{8}$$

$$a_{X^-/2}\, a_{M^+/2} = a_{X^-/1}\, a_{M^+/1} \tag{9}$$

$$a_{X^-/2}\, a_{H^+/2} = a_{X^-/1}\, a_{H^+/1} \tag{10}$$

$$a_{M^+/2}\, a_{OH^-/2} = a_{M^+/1}\, a_{OH^-/1} \tag{11}$$

$$a_{H^+/2}\, a_{OH^-/2} = a_{H^+/1}\, a_{OH^-/1} \tag{12}$$

There are four more equations than the number of variables. It is possible to eliminate four equations: e.g. (7, 8, 11 and 12). Only for algebraic simplicity we shall substitute activities (a_{y_i}) by molal concentrations (m_{y_i}). From equations (1, 2, 9 and 10) the following series of equations was obtained:

$$\frac{m_{M^+/1}}{m_{M^+/2}} = \frac{m_{X^-/12}}{m_{X^-/1}} = \frac{m_{H^+/1}}{m_{H^+/2}} = \frac{m_{OH^-/2}}{m_{OH^-/1}} \tag{13}$$

Substituting the expressions shown in Fig. 3 we obtain:

$$\frac{(K_w/y) + n + zm_{p\text{-}z} - y}{(K_w/y) + m - x} = \frac{m}{n} = \frac{y}{x} = \frac{K_w/x}{K_w/y} = \lambda \tag{14}$$

which can be rearranged to obtain:

$$\frac{(K_w/y) + n + zm_{p\text{-}z}}{(K_w/x) + m_{p\text{-}z}} = \lambda \tag{15}$$

Substituting in (15) the following expressions derived from (14):

$$y = \lambda x \tag{16}$$

$$m = \lambda n \tag{17}$$

we obtain:

$$\lambda = \sqrt{1 + \frac{zm_{p\text{-}z}}{(K_w/y) + n}} \cong \sqrt{1 + \frac{zm_{p\text{-}z}}{n}} \tag{18}$$

This expression gives the distribution constant λ for all permeable solutes in terms of the number of charges and the concentration of the charged macromolecule, the proton and the anion concentration, all in side 1. The approximation is valid under the pH and electrolyte concentrations used in this study. The final expression is obtained by noting that by definition (Eq. 14) log λ represents the pH difference between sides 1 and 2, i.e., between coated surface and food bulk in the case of our intended application. From Eq. (16) we obtain:

$$\log \lambda = \log y - \log x = \text{pH (food bulk)} - \text{pH (coating)} \quad (19)$$

APPLICATIONS TO AN IMF MODEL

IMF Model Requirements

The key parameters in the expression for the pH difference between coated surface and food bulk are: electrolyte concentration and the number of immobilized charged groups on the surface. This is further emphasized in Table 1, which reports the calculated pH difference as affected by these two parameters and shows that significant ΔpH values can only be achieved in an IMF system with low electrolyte content. To prove the validity and potential application of the pH difference concept, an IMF model has to satisfy this condition.

Selection of a Charged Macromolecule

The ideal polyelectrolyte should have a large number of strongly dissociated groups. Solubility and ease of application should also be considered. Several food grade polyelectrolytes satisfy these conditions, including pectic acid, xanthan gum, furcellaran and carrageenans.

MATERIALS AND METHODS

The IMF model chosen to test the feasibility of the surface pH modification was an IM cheese analog developed in this laboratory (Torres *et al.* 1985; Motoki *et al.* 1982). To reduce total electrolyte content this product was reformulated as shown in Table 2. Glycerol (Certified A.C.S., Fisher Scientific Co., Fair Lawn, NJ) and sorbitol (Pfizer Co., New York, NY) were dissolved in warm water (about 50°C) with sorbic acid (Pfizer Co.) as the mycostatic agent. Electrolytes present in the proteins used, isolated

Table 1. ΔpH values as affected by the concentration of charged groups and the concentration of electrolytes present in a food product

Charged groups, [M] $zm_{p-z,}$ [a]	Electrolytes, [M] n	$\log\lambda = \Delta pH$
0.001	0.0100	0.02
	0.0010	0.21
	0.0001	1.00
0.010	0.0100	0.21
	0.0010	1.00
	0.0001	2.00

[a] equivalent to a macromolecule with z = −10 and a concentration ranging from 0.0001 to 0.001M, i.e. a molecule with m.w. = 10,000 used in the 1 to 10% range.

Table 2. Composition of the low electrolyte IMF model

Component	Amount, g
Isolated soy protein	26.1
Caseinates	7.9
Hydrogenated vegetable oil	34.0
Emulsifier	1.6
Glycerol	5.9
Sorbitol	47.0
Sorbic acid	0.4
Water[a]	100 to 56.2

[a] Moisture content reduced by air drying (Motoki *et al.* 1982).

soybean protein (ISP, Ajinomoto USA Co., New York, NY) and sodium and calcium caseinates (Erie Casein Co., Erie, IL), were eliminated by dialysis. Electrolyte removal was followed by electrical conductivity measurements (Model 10 conductimeter, Markson Science, Del Mar, CA). A NaCl standard curve was used to estimate electrolyte concentrations from electrolyte conductivity measurements. Measurements on the protein solutions themselves were not possible because proteins are conductive. Permeate measurements were used instead. The rest of the model preparation was as reported previously.

Table 1 shows that significant pH differences between bulk food and treated surface are possible only when the total electrolyte concentration is kept below approximately 0.001M. This restriction was used to estimate protein dialysis requirements as follows. Protein concentration in the model system is 46.4 g/100 g water while in the dialysis experiment it was 7 g/100 g water. Assuming linearity between conductivity measurements and electrolyte concentration, an upper level of 0.001M represents a limit of 0.00015 M in the dialysis experiment.

Three ISP batches were prepared, each consisting of a 7 g/l (100 g total protein) solution dialyzed against 20 l deionized distilled water, which was changed as frequently as required. Batch A was an ISP solution dialyzed as is for 7 days at 4°C. Batch B was a solution adjusted to pH 8, dialyzed for 4 days at 4°C and then neutralized by dialysis against 0.0001 M HCl. Batch C was an ISP solution dialyzed as is for 12 h at 60°C. 7 g/l (50 g total protein) Na- and Ca- caseinates solutions were dialyzed against 6 l deionized distilled water for 4 days at 4°C. Dialyzed protein solutions were steamed for 15 min and then freeze dried. IMF samples were labelled according to the ISP dialysis method.

The polyelectrolyte chosen for experimental tests was λ-carrageenan (Sigma Chemical Co., St. Louis, MO) incorporated in an agarose gel (Gel Electrophoresis Grade, BRL, Gaithersburg, MD) shown in Table 3. This polyelectrolyte was chosen for its solubility and high percentage of sulfate groups — a strongly dissociated group (sulfuric acid dissociation constants: K_1 = large, K_2 = 1.2 x10^{-2}; Weast 1972, page D-121). Agarose was chosen as the coating forming matrix for its lack of charged groups which explains why its gelling properties are independent of pH and salt concentration, important considerations in our study.

Best coatings were obtained when agarose was steamed for 30 min. Therefore, λ-carrageenan could not be added to the formulation until after heating. Sulfate groups can cause agarose- and self-hydrolysis as detected in preliminary experiments. Although 2 h in a water bath at 85°C did not affect sorbic acid stability significantly (Torres and Karel 1984a), it is safer to add it after heating, immediately before use. As with

Table 3. Composition of the λ-carrageenan coating

Component	Amount, g
Deionized agarose	1.0
Deionized carrageenan	1.0
Sorbic acid	1.2
Propylene glycol, 40% aq. soln.	96.8

proteins, dialysis was used to eliminate electrolytes normally present in commercial reagent grade λ-carrageenan and agarose. 1% solutions were dialyzed for 2 days at 4°C and then freeze dried.

About 5 g IMF samples were pressed into sterile 35 mm diameter disposable Petri dishes working under a laminar air flow hood. About 0.5 ml of the λ-carrageenan/agarose mixture was poured on top. Samples were then stored at 35°C and 88% RH (over saturated $BaCl_2$. Samples were then removed periodically and the pH difference between coated top and uncoated bottom was measured with the surface pH electrode.

RESULTS AND DISCUSSIONS

Dialysis experiments yielded fractions with the characteristics summarized in Table 4. The three dialysis conditions yielded ISP fractions with similar electrolyte levels. This information, composition data (Tables 2 and 3) and Eq. (17 and 18) were used to estimate that IMF samples would have a food surface/food bulk pH difference of approximately 0.8 pH units.

Experimentally a constant pH difference was not achieved instantaneously (Fig. 5a, batch A, runs 1 and 2). About 3 to 4 days were needed to reach stable conditions. The final pH difference was 0.3 to 0.5 pH units, i.e., somewhat lower than the calculated value: 0.8. The difference most probably due to our assumption that the only electrolyte sources are the protein fractions.

In Fig. 5a we have also represented the calculated percent of undissociated sorbic acid (pK_a = 4.8) corresponding to bulk and localized reduced surface pH conditions. The difference between these two curves visualizes the strong microbial stabilizing effect that can be achieved by reducing

Table 4. Electrolyte elimination by dialysis final permeate conductivity measurements

Ingredient	Conductivity [micromho]	Equivalent NaCl [M] x 10^{-3}
ISP, batch A	30 - 40	3 - 4
batch B	20	2.0
batch C	18	1.8
Na-caseinate	2	0.2
Ca-caseinate	2	0.2
λ-carrageenan	<<2	<<0.2

surface pH. The pH conditions established on the surface have more than doubled the concentration of the undissociated, i.e., the active, form of the preservative.

It should be noted that bulk acidification is not always feasible. In most products it will adversely affect its organoleptic acceptability (e.g., Motoki *et al.* 1982). This incompatibility between enhanced microbial stability and organoleptic quality, typical of IMF technology limitations, could be solved, in some cases, with surface pH reduction.

ISP from batches B and C were used for longer testing periods with the ΔpH values (after day 4) shown in Fig. 5b. Based on conductivity measurements the similarity to Fig. 5a was expected, and confirms the prediction power of the Donnan equilibrium model.

As indicated the pH difference disappeared 25 days after surface treatment. At the same time, growth of unidentified bacteria and fungi was observed. Multiple samples taken at day 31 confirmed this finding. As shown in Fig. 5 stable pH conditions had already been established, therefore the most likely explanation has to be microorganisms contaminating the model during preparation. It should be noted that at testing conditions food bulk is by design not stable. Only the surface is affected by the proposed treatment.

Additional studies also reported (Torres and Karel 1985) showed that surface pH reduction increased surface microbial stability. Under conditions allowing microbial outgrowth, a_w = 0.88, RH = 88%, T = 35°C, %sorbic acid = 0.22, samples challenged with *Staphylococcus aureus* S-6 were stable for 20 or more days.

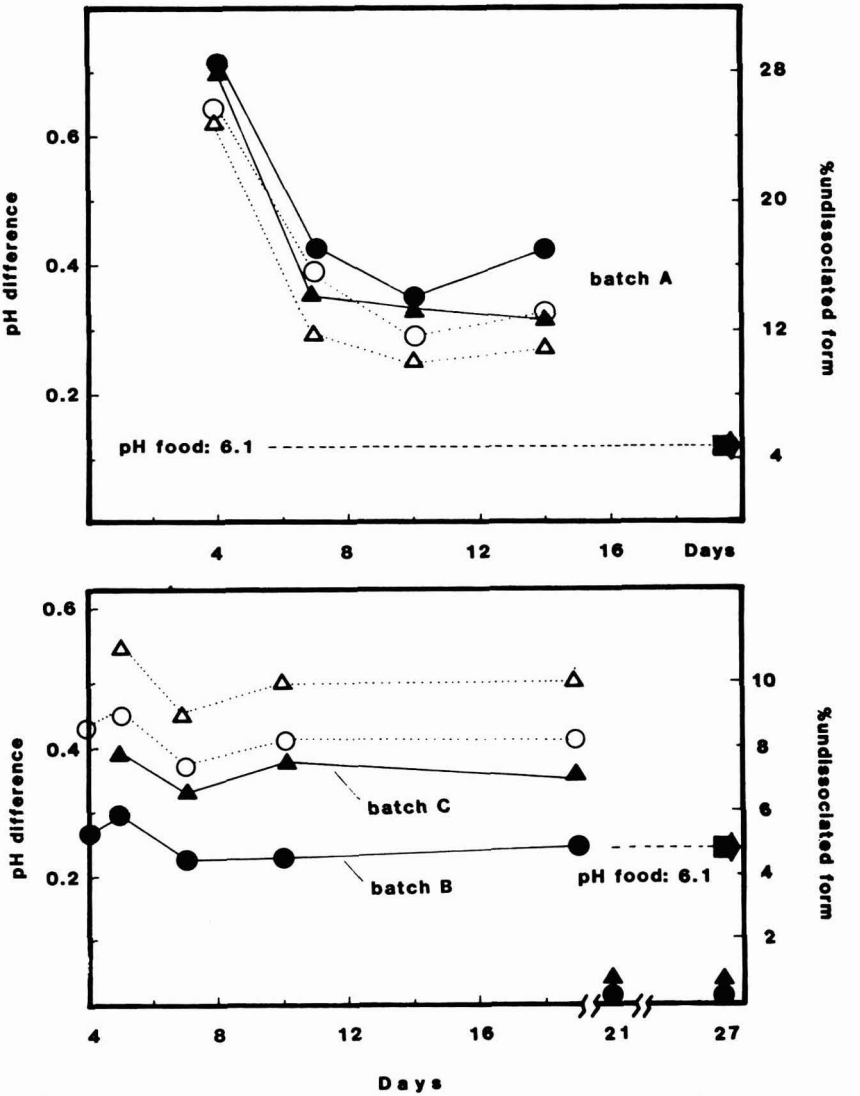

FIG. 5. pH DIFFERENTIAL ESTABLISHED BY THE APPLICATION OF A λ-CARRAGEENAN/AGAROSE COATING ON THE REDUCED ELECTROLYTE CONCENTRATION IMF MODEL, AND CALCULATED EFFECT ON THE AVAILABILITY OF UNDISSOCIATED SORBIC ACID

a. Runs 1 [●, ○] and 2 [▲, △] with ISP batch A.
[●, ▲]: measured pH
[○, △]: calculated % active form of the sorbic acid at the surface pH conditions
[---➤]: calculated % active form of sorbic acid at food bulk pH conditions (6.1)

b. ISP batches B [●, ○] and C [▲, △].
[●, ▲]: measured pH
[○, △]: calculated % active form of the sorbic acid at the surface pH conditions
[---➤]: calculated % active form of sorbic acid at food bulk pH conditions (6.1).

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REFERENCES

AKEDO, M., SINSKEY, A. J. and GOMEZ, R. 1977. Antimicrobial action of aliphatic diols and their esters. J. Food Science *42*(3), 699.

ANONYMOUS. 1972. Broad line of intermediate moisture foods approaching reality. Food of Tomorrow, Autumn 1972, Supplement to Food Processing *33* (10),14.

BHATIA, B. S. and MUDAHAR, G. S. 1982. Preparation and storage studies of some intermediate moisture vegetables. J. Food Science and Technology (India) *19*(1),40.

DONNAN, F. G. 1934. Die genaue Thermodynamik der Membrangleichgewichte. II. Z. Physikal Chem. Abt. A *168*(516),24.

DONNAN, F. G. 1924. The theory of membrane equilibria. Chemical Reviews *1,*73.

DONNAN, F. G. 1911. Theorie der Membrangleichgewichte und Membranpotentiale bei Vorhandesein von nicht dialysierenden Elektrolyten, ein Beitrage zur Physikalicsh-Chemisch Physiologie. Zeitschrift fur Elektrochemi *17,*572.

DONNAN, F. G. and GUGGENHEIM, E. A. 1932. Die genaue Thermodynamik der Membrangleichgewichte. I. Z. Physical Chem. Abt. A *162,*346.

EKLUND, T. 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J. Appl. Bacteriology *54;*383.

ERICKSON, L. E. 1982. Recent developments in intermediate moisture foods. J. Food Protection *45*(5),484.

FLORA, L. F., BEUCHAT, L. R. and RAO, V. N. M. 1979. Preparation of a shelf stable food product from muscadine grape skins. J. Food Science *44*(3),854.

FREESE, E., SHEU, C. W. and GALLIERS, E. 1973. Function of lipophilic acids as antimicrobial food additives. Nature *241,*321.

GRIGNON, J. and SCALLAN, A. M. 1980. Effect of pH and neutral salts upon the swelling of cellulose gels. J. Applied Polymer Science *25,*2829.

GRUNDKE, G. and KUKLOV, K. 1980. Das Kryptoklima in Lebensmittelver-packungen. Lebensmittelindustrie *27*(1),13.

HANSEMAN, J. Y., GUILBERT, S., RICHARD, N. and CHEFTEL, J. C. 1980. Influence conjointe de l'activite de l'eau et du pH sur la croissance de *Staphylococcus aureus* dans un aliment canne a humidite intermediaire. Lebensm. -Wiss. und -Technologie *13,*269.

HIEMENZ, P. C. 1977. Osmotic and Donnan equilibrium. Ch.4. In *Principles of Colloid and Surface Chemistry.* 1st Edition, p. 125. Marcel Dekker, Inc., New York.

HSU, H. W., DENG, J. C., KOBURGER, J. A. and CORNELL, J. A. 1983. Storage stability of intermediate moisture mullet roe. J. Food Science *48,*172.

LEISTNER, L. and RODEL, W. 1976. The stability of intermediate moisture foods with respect to microorganisms. In *Intermediate Moisture Foods,* (R. Davies, G. G. Birch and K. J. Parker, eds.), p. 20. Applied Science Publishers Ltd., London.

MOTOKI, M., TORRES, J. A. and KAREL, M. 1982. Development and stability of intermediate moisture cheese analogs from isolated soybean proteins. J. Food Proc. Pres. *6*(1),41.

PAVEY, R. L. 1972. Controlling the amount of internal aqueous solution in intermediate moisture foods. Swift and Co., Res. Development Center, Oak Brook, Ill. Contract No. DAAG 17-70-C-0077, Food Laboratory, U.S. Army Natick Laboratories, MA 01760.

SCALLAN, A. M. and GRIGNON, J. 1979. The effect of cations on pulp and paper properties. Svensk papperstidning *2,*40.

THERON, D. P. and PRIOR, B. A. 1980. Effect of water activity and temperature on *Staphylococcus aureus* growth and thermonuclease production in smoked snoek. J. Food Protection *43,*370.

TORRES, J. A. 1984. Exploration of the stability of intermediate moisture foods. Ph.D. thesis, Massachusetts Institute of Technology, Cambridge.

TORRES, J. A. and KAREL, M. 1984a. Sorbic acid stability during processing and storage of an intermediate moisture cheese analog. J. Food Science (submitted for publication).

TORRES, J. A. and KAREL, M. 1985. Microbial stabilization of intermediate moisture food surfaces. III. Effects of surface pH control and surface preservative concentration on microbial stability of an intermediate moisture cheese analog. J. Food Proc. Pres. *9,*00.

TORRES, J. A., MOTOKI, M. and KAREL, M. 1985. Microbial stabilization of intermediate moisture food surfaces. I. Control of preservative concentration. J. Food Proc. Pres. *9,*00.

WEAST, R. C. (editor) 1972. *Handbook of Chemistry and Physics,* 53rd Edition, The Chemical Rubber Co., Cleveland, Ohio.

MICROBIAL STABILIZATION OF INTERMEDIATE MOISTURE FOOD SURFACES
III. EFFECTS OF SURFACE PRESERVATIVE CONCENTRATION AND SURFACE pH CONTROL ON MICROBIAL STABILITY OF AN INTERMEDIATE MOISTURE CHEESE ANALOG

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ABSTRACT

Localized surface condensations affect microbial stability of intermediate moisture foods (IMF). Two surface modifications previously reported were tested for their ability to improve microbial surface stability; control of surface preservative concentration and reduced surface pH.

The effectiveness of a high surface sorbic acid concentration controlled by a zein coating was confirmed in Staphylococcus aureus S-6 surface challenge experiments under extreme testing conditions. Samples with bulk water activity (a_w) of 0.88, stored at 30°C under constant 88% relative humidity (RH) remained stable for over 16 days. Uncoated controls were stable for only 2 days. Samples with bulk a_w = 0.85, exposed to cycles of 12 h at 85% RH and 12h at 88% RH, remained stable for more than 28 days. Uncoated controls were stable for only 3 days.

The reduced surface pH approach to microbial stability required the formulation of an IMF model with low total electrolyte concentration which was coated with a deionized mixture of λ-carrageenan and agarose. The effectiveness of the resulting 2.5 fold increase in the surface availability of the active form of sorbic acid as compared to food bulk was confirmed in a challenge test with S. aureus S-6. In a test with samples at a_w = 0.88, stored at RH = 88% and 30°C, we found a stability period of about 20 days and it seems possible to increase it.

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INTRODUCTION

One of the problems in the development of commercial IMF is the difficulty in achieving the desired water activity (a_w) and at the same time satisfying the many organoleptic and safety restrictions (Torres *et al.* 1984a; Troller and Christian 1978). Moreover, once a formulation is found to satisfy a design criteria, it is not always possible to include safety margins protecting against abuse. This is particularly true of the changing temperature conditions common to food production and distribution. An analysis of these situations (Torres 1984) showed that they result in localized surface condensations. These moisture gains disrupt the delicate balance provided to maintain microbial stability and surface microbial growth is often observed. This possibility has been mostly ignored by researchers working on this field, who have tested IMF's under conditions of constant temperature and humidity conditions (e.g., Hsu *et al.* 1983; Bhatia and Mudahar 1982; Erickson 1982; Theron and Prior 1980; Hanseman *et al.* 1980; Flora *et al.* 1979; Pavey 1972; Anonymous 1972). The approach in our studies (Torres *et al.* 1985a, b) has been to consider the surface as a separate region whose stability has to be considered independently from the bulk of the product, and to develop treatments that would enhance surface microbial stability. The modified properties should inhibit, or at least reduce, microbial growth to such an extent that water diffusion into the food bulk will occur before high cell concentrations are achieved.

We explore two approaches for improving surface stability: (1) Use of high concentrations of preservative on the surface. A coating maintains this concentration difference for as long as possible. The preservative selected for our studies was sorbic acid and its potassium salt; the coating found to satisfy our requirements was an ethanol zein solution (Torres *et al.* 1985a). The IMF model used (IMF model No. 1) was an IM cheese analog previously developed in our laboratories (Motoki *et al.* 1982). (2) Create a surface microenvironment where pH is lower than that of the food bulk. Most food preservatives, particularly sorbic acid, are lipophilic acids whose effectiveness is pH dependent (Eklund 1983; Motoki *et al.* 1982; Akedo *et al.* 1977; Freese *et al.* 1973). A λ-carrageenan/agarose based coating can be used to establish a permanent pH difference, 0.3 to 0.5 pH units, when applied on an IMF model with low total electrolyte concentration, about 0.005M (Torres *et al.* 1985b). This reduction was used to predict a 2.5 fold increase in the availability of the active undissociated form of the preservative as compared to bulk food pH conditions, 6.1. The IMF model was the IM cheese analog modified to reduce total electrolyte concentration (IMF model No. 2).

This paper reports microbial surface challenge studies, under extreme testing conditions, on IM samples with the surface treatments described above.

MATERIALS AND METHODS

Controlled Surface Preservative Concentration Sample Preparation

Table 1 shows the component of a cheese analog, IMF model No. 1, whose preparation has been reported previously (Motoki *et al.* 1982; Torres *et al.* 1985a). The initial water content is 100ml/100g solids and is reduced to the desired a_w by blowing warm air into the food cutter bowl. The mixture was then stuffed into a cellulose casing (Type 30F0, Union Carbide, Tarrytown, NY) with a hand press. Thereafter, casings were placed into seamless polyvinylidene chloride casing tubes (diameter 40 mm, Kreha Chemical Co., Tokyo, Japan) and pasteurized for 2 h in a water bath kept at 85°C. A long heating period was used to eliminate all vegetative cells present in the ingredients or accumulated during the long preparation procedure.

Table 1. Composition of IMF model No. 1[a]

INGREDIENT	
Isolated soy protein	26.1
Na-caseinate	5.9
Ca-caseinate	2.0
Hydrogenated vegetable oil	34.0
Decaglycerol monooleate	0.4
Salt	4.8
Glycerol	5.9
Sorbitol	19.3
K-sorbate	variable

[a] The initial water content is 100 ml/100g solids and is reduced to the desired a_w by blowing warm air into the food cutter bowl (Motoki *et al.* 1982).

After cooling, pH was determined with a surface electrode probe (combination electrode 39507, Beckman Instruments, Inc., Cedar Grove, NJ). Casings were then carefully removed under a laminar air flow hood and the cylinder thus obtained was cut into disks. Each disk was placed on a sterile dissecting needle and stored in a constant RH chamber, maintained at 88 or 85% RH by the use of saturated solutions, $BaCl_2$ or $Sr(N0_3)_2$, respectively. The chamber itself was kept in circulation by the use of a fan. Zein ethanol solutions with the composition given in Table 2 were used to prepare spray-coated samples. Uncoated samples were separated for control purposes. To avoid artifacts caused by residual ethanol, samples were stored in the constant humidity chamber for at least twelve hours. Thereafter, coated and uncoated samples received a surface application of sorbic acid by spraying them with a 10% preservative solution in ethanol (190 proof) equivalent to an additional 0.2% K-sorbate. Spraying conditions were determined experimentally as follows: several samples were coated with zein and then sprayed with the sorbic acid solution. Sorbic acid determinations were represented then as a function of spraying time. The sprayer used was hand held 125 ml glass atomizer (Fisher Scientific, Pittsborough, PA). To eliminate residual ethanol samples were stored in the constant RH chamber for at least another twelve hours. Various samples were used to determine whether a_w changes had occurred using an electric hygrometer (SINA Equihygroscope, distributed by Beckman Instruments Inc.).

S. aureus S-6 stocks used in the microbial challenge studies were kept in brain heart infusion (BHI, Difco, Detroit, MI) agar tube slants at refrigeration temperature. Fresh cultures were prepared approximately every two weeks. An inoculation stock was prepared by transferring cells from the

Table 2. Zein coating solution

Component	%, w/w
Zein[a]	x
Glycerol	0.25 x
Myvacet	1
ethanol, 190 proof	(balance)

[a] Acetylated monoglycerides (Eastmen Kodak Co., Rochester, NY).

slants to BHI broth. After incubation at 30°C to mid-log phase (approximately 120-160 Klett units) the broth was diluted 100 times with 40% glycerol to reduce a_w and cell concentration. Samples were dipped in this cell suspension, then placed in individual Petri dishes and stored in dessicators at 85 or 88% RH and 30°C. For control purposes some samples were not inoculated. Preparation of media, dilution and plating procedures were according to standard microbiological methods (AOAC 1976). A procedure to determine location of growth was included to determine if it was possible to assume that the bulk of inoculated samples remained sterile, and therefore cell numbers obtained could be expressed as surface growth, i.e., as cells/cm^2.

Samples were used in the following studies: (1) Not inoculated samples after various surface treatments were used to determine background microbial load, i.e., final sample sterility. All samples were stabilized with 0.1% K-sorbate. Some samples received a surface application equivalent to an additional 0.2%. Bulk a_w was 0.88 and storage conditions were 88% RH and 30°C. (2) Inoculated samples, with various surface treatments and bulk a_w 0.88, were stored at 30°C and 88% RH. Samples that had shown 3 to 4 log cycles increases above inoculation level, i.e., after 11 days, were peeled to separate a surface and a core fraction. All samples contained 0.1% K-sorbate. In addition some samples received a surface application equivalent to an additional 0.2%. Separate counts were determined for the surface and the core fraction to determine growth location. (3) Inoculated, uncoated samples with sorbic acid added to the surface were used as positive controls. Information on samples without surface sorbic acid has already been published (Motoki *et al.* 1982). (4) Inoculated, zein coated samples with sorbic acid added to the surface were used to determine coating effectiveness. Two sets of experiments will be reported. In the first one, samples with bulk $a_w = 0.88$ were stored at 30°C and 88% RH, and in the second, samples with bulk $a_w = 0.85$ were stored at 30°C and exposed to 12 h cycles of 85 and 88% RH [over saturated $Sr(N0_3)_2$ and $BaCl_2$, respectively]. Based on the paper by Motoki *et al.* (1982) we knew that $a_w = 0.88$ allowed rapid *S. aureus* outgrowth when only 0.3% K-sorbate was present.

Reduced Surface pH Sample Preparation

The IM cheese analog was modified to test the surface microbial stability enhancement of a λ-carrageenan/agarose coating previously described (Torres *et al.* 1985b). Elimination of electrolytes resulted in the composition given in Table 3, with a total electrolyte content approximately equal to 0.005M. About 5 g of pasteurized sample, prepared as described by Torres *et al.* (1985b) were pressed into sterile 35 mm diameter disposable

Table 3. Composition of IMF model No. 2

Component	Amount, g
Isolated soy protein, deionized [a]	26.1
Caseinates, deionized	7.9
Hydrogenated vegetable oil	34.0
Emulsifier	1.6
Glycerol	5.9
Sorbitol	47.0
Sorbic acid	0.4
Water[b]	

[a] Three deionized ISP batches were prepared as reported by Torres *et al.* (1984b) and labelled A, B and C.

[b] The initial water content is 100 ml/100g solids and is reduced to the desired a_w by blowing warm air into the foot cutter bowl (Motoki *et al.* 1982).

Table 4. λ-Carrageenan coating composition

Component	%, w/w
Agarose, deionized	1.0
λ-carrageenan, deionized	1.0
Sorbic acid	1.2
Propylene glycol, 40% aq. soln.	(balance)

Petri dishes, working under a laminar air flow hood. About 0.5 ml of a λ-carrageenan/agarose with the composition given in Table 4 was poured on top. After coating gelling and a ΔpH equilibration period of about 4 days (Torres *et al.* 1985b) samples were inoculated with 0.1 ml of a *S. aureus* cell suspension at a_w 0.88 and then stored at 30°C and 88% RH (over saturated $BaCl_2$). Samples were removed periodically for microbial count determinations following procedures described previously. Three IMF sample runs were prepared and labelled according to the isolated soybean protein (ISP) fraction used, i.e., A, B and C. The preparation of these ISP fractions have been reported previously (Torres *et al.* 1985b).

EXPERIMENTAL RESULTS

Controlled Surface Preservative Concentration

Background microbial load tests for various surface treatments have been summarized in Table 5. Cell counts did reach detectable counts after approximately a week storage for uncoated samples and only much later (2 to 3 weeks or more) for coated samples. The difference seems to indicate that sample cores were free of contaminants and that surface contamination occurred during sample preparation and/or sample removal. Since the surface of coated samples had enhanced growth inhibiting properties, it is to be expected that they will have lower counts for longer periods of time.

Coated and uncoated inoculated samples that had shown 3 to 4 log cycles increases above inoculation level, were used to determine if growth was occurring only on the surface of food samples. Table 6 shows that it is safe to assume that growth is limited to sample surface. Significant cell numbers were detected in the core, but they represent most probably an experimental artifact. It is very difficult to separate a sterile core from a heavily contaminated surface without cross-contamination. This operation was slightly more difficult for coated samples due to the mechanically stronger zein coating.

Coating effectiveness was determined first in tests at high relative humidity. These tests correspond to samples with bulk $a_w = 0.88$, stored at 30°C and constant 88% RH. To allow for comparisons between different treatments a stability limit was defined as follows: samples are no longer considered stable if cell counts are one cycle above inoculation level. At this point, it is important to mention that cell numbers, reported as time zero inoculation levels, were obtained about 4 h after inoculation (Fig. 1). That is why, inoculation levels for effective surface treatments, those that

Table 5. Microbial background load tests: number of days before not-inoculated samples reached detectable counts[a]

Treatment	days
Uncoated	6.5
Uncoated w/ .2% sorbic acid added as dip	6.5
Coated w/ 3 20% zein solution dips w/ .2% sorbic acid added as spray	16.0
Coated w/ 3 12% zein solution dips w/ .2% sorbic acid added as spray	16.0

[a] About 1,000 cells/cm^2

even show initial bactericidal effects, are lower than those obtained for positive controls. Therefore we have chosen as initial inoculation values those measured for positive controls.

Figure 1a shows the effectiveness of samples coated by dipping in 20% zein solutions. The stability period can be estimated as being between 5 and 8 days. There is also a significant bactericidal effect during the initial storage period. Figure 1b shows the stability tests of samples coated by spraying them 3 times with 12% zein solutions. The stability period has increased to about 10 to 16 days.

Table 6. Location of growth tests: comparison of surface and core fraction counts

Treatment	Surface counts x 10^7	Core counts x 10^5
Uncoated	6.7	2.6
Uncoated w/ .2% sorbic acid as dip	5.6	0.1
Coated w/ 8-12-20% zein dips w/ .2% sorbic acid as dip	24.0	140.0
Coated w/ 3 12% zein dips w/ .2% sorbic acid as dip	16.0	25.0

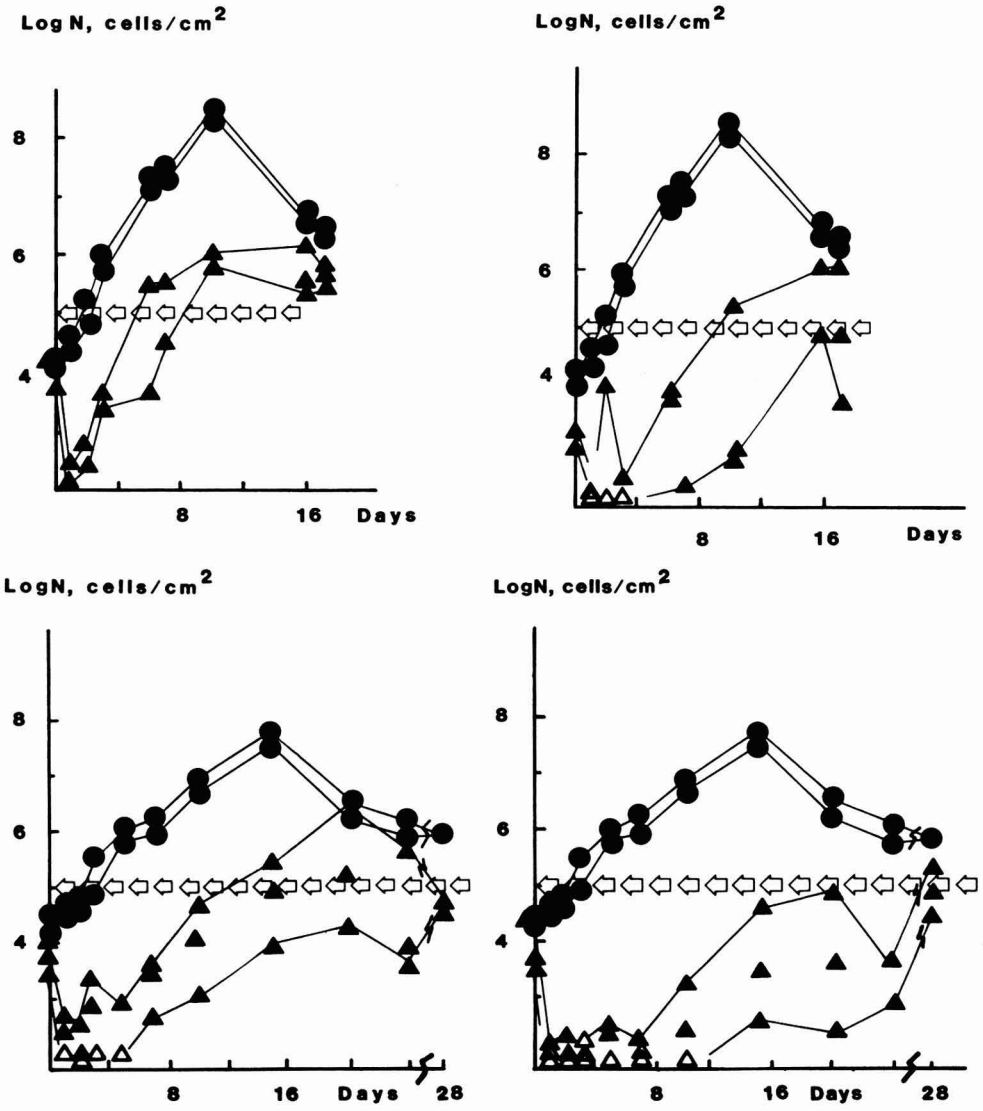

FIG. 1. STABILITY TESTS Samples were taken in duplicates or triplicates and individual determinations have been plotted as a function of time. Lines have been drawn through maximum and minimum values. The horizontal line represents the stability limit defined in the text. Empty symbols are used to indicate whenever no counts were detected, i.e. under 1,000 counts/cm^2.

[▲ △]: coated samples; [●]: uncoated controls (a.) zein dip coated samples, bulk a_w = 0.88, challenged with *Staphylococcus aureus* S-6. Storage at 30°C under constant 88% RH conditions. (b.) zein spray coated samples, bulk a_w = 0.88, challenged with *Staphylococcus aureus* S-6. Storage at 30°C under constant 88% RH conditions. (c.) zein dip coated samples, bulk a_w = 0.85, challenged with *Staphylococcus aureus* S-6. Storage at 30°C under cycling RH conditions, 12 hours at 88 and 12 hours at 85% RH. (d.) zein spray coated samples, bulk a_w = 0.85, challenged with *Staphylococcus aureus* S-6. Storage at 30°C under cycling RH conditions, 12 hours at 88 and 12 hours at 85% RH.

Coating effectiveness was also determined in tests with cycles of low and high relative humidity. Samples with bulk a_w = 0.85, stored at 30°C, were exposed to cycles of 12 h at 85% RH and 12 h at 88% RH. Figure 1c shows the effectiveness of samples coated with 20% zein dips, a repetition of preparation conditions reported in Fig. 1a. The only difference was that storage conditions tested were less demanding. The change in bulk a_w and storage RH conditions resulted in an increased stability period: 10 to 15 days. Finally, Fig. 1d shows the stability tests of samples sprayed with 12% zein exposed to the RH cycle. In this case the stability period seems to be longer than, or about the length of the testing periods, 28 days.

Further analysis of the microbial tests reported here indicate that spraying of zein solutions on food samples is a more effective treatment than dipping. The exact nature of this difference was not determined.

Microbial tests showed large differences between individual experimental replicates. The cause behind this variation has been traced to variability in the amount of sorbic acid deposited on coated surfaces (Torres and Karel 1984).

Samples with bulk a_w = 0.88 and pH = 6.4, stored at constant 88% RH and 30°C represent extreme testing conditions (Motoki *et al.* 1982). Such conditions were chosen to accelerate testing time.

Samples with lower bulk a_w, 0.85, challenged with cycles of low and high RH, 85 to 88%, RH, 12 h cycles, resulted in significantly longer stability periods. This cycling test was an approximation to situations found in commercial distribution of IMF's. Unfortunately, no model is available to extrapolate from these results, the consequences of other abuse conditions. Only qualitative comments are possible.

The severity of the above tests should not be underestimated. Surface challenge conditions, in terms of a_w, pH and temperature were capable to support outgrowth of *S. aureus* (Motoki *et al.* 1982). The only hurdle was the high concentrations of sorbic acid existing on the surface of coated samples.

Reduced Surface pH

The use of reduced surface pH conditions required the development of a mathematical model based on the Donnan equilibrium theory for semipermeable membranes. This model was capable of predicting the experimental requirements for reduced surface pH conditions: low total electrolyte concentration and a coating immobilizing a large polyelectrolyte (Torres *et al.* 1985b).

After pH equilibration, samples were inoculated with *S. aureus* S-6. Inoculated uncoated samples were used as positive controls. Figure 2

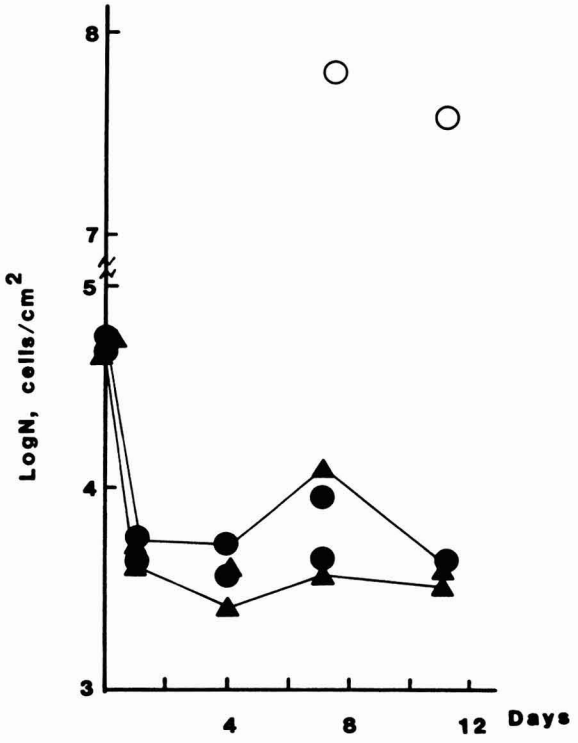

FIG. 2. STABILITY TEST: EFFECT OF REDUCED SURFACE pH λ-carrageenan/ agarose coated samples, bulk a_w = 0.88, challenged with *Staphylococcus aureus* S-6. Storage at 30°C under constant 88% RH conditions. Experiment was run twice in duplicates [●, ▲]. Lines have been drawn through maximum and minimum values and should be compared with uncoated controls. [○]

shows the results from batch A and visualizes the strong stabilizing effect of reduced surface pH. We have estimated that as a result of the treatment the availability of undissociated sorbic acid, the effective form of the preservative, is 2.5 times higher on the surface than in the food bulk (Torres *et al.* 1985b). ISP from batches B and C were used for more extended microbial tests. Growth occurred only after 21 days after inoculation, i.e., 25 days after surface treatment. Growth of unidentified bacteria and fungi was observed. At the same time, the pH difference disappeared. Multiple samples taken at day 27 confirm this finding. The observation could not be interpreted as a ΔpH disappearance caused by a "pH equilibration" between surface and food bulk (Torres *et al.* 1985b). Most likely, the explanation lies in unavoidable microbial contamination collected during the long sample preparation process, particularly while pressing samples into Petri dishes under the laminar air flow hood. It should be noted that at testing conditions food bulk is not stable, only the surface is affected by our treatment.

Note also that extreme testing conditions were used: bulk a_w = 0.88, 88% RH, bulk pH = 6.1, storage temperature = 30°C and sorbic acid = 0.22% (w/w). The only hurdle to microbial growth was the reduced surface pH condition.

CONCLUSIONS

Improved microbial stability was achieved when zein coatings were applied to IMF model No. 1. This result is consistent with predictions based on permeability cell experiments and sorbic acid distribution studies previously reported (Torres *et al.* 1985a). It is important to note that the successful challenge studies reported here utilized coatings only 0.03 to 0.04 mm thick (Torres *et al.* 1985a). It remains to be determined what the maximum organoleptically acceptable value could be.

Coating a low electrolyte IMF model with a λ-carrageenan/agarose coating improved microbial stability as predicted from pH measurements previously reported (Torres *et al.* 1985b). The possibility of increasing polyelectrolyte concentration to overcome the need to reduce electrolyte concentration, at least to some extent, will be explored in future studies.

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REFERENCES

AKEDO, M., SINSKEY, A. J. and GOMEZ, R. 1977. Antimicrobial action of aliphatic diols and their esters. J. Food Science *42*(3),699.

ANONYMOUS. 1972. Broad line of intermediate moisture foods approaching reality. Foods of Tomorrow, Autumn 1972. Supplement to Food Processing *33*(10),14.

AOAC. 1976. FDA Bacteriological Analytical Manual for Foods. Association of Official Analytical Chemists, Washington, D.C.

BHATIA, B. S. and MUDAHAR, G. S. 1982. Preparation and storage studies of some intermediate moisture vegetables. J. Food Science and Technology (India) *19*(10),40.

EKLUND, T. 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J. Appl. Bacteriology *54*,383.

ERICKSON, L. E. 1982. Recent development in intermediate moisture foods. J. Food Protection *45*(5),484.

FLORA, L. F., BEUCHAT, L. R. and RAO, V. N. M. 1979. Preparation of a shelf stable food product from muscadine grape skins. J. Food Science *44*(3),854.

FREESE, E., SHEU, C. W. and GALLIERS, E. 1973. Function of lipophilic acids as antimicrobial food additives. Nature *241*,321.

HANSEMAN, J. Y., GUILBERT, S., RICHARD, N. and CHEFTEL, J. C. 1980. Influence conjointe de l-activite de l'eau et du pH sur la croissance de *Staphylococcus aureus* dans un aliment canne a humidite intermediaire. Lebensm. -Wiss. und -Technologie *13*,269.

HSU, H. W., DENG, J. C., KOBURGER, J. A. and CORNELL, J. A. 1983. Storage stability of intermediate moisture mullet roe. J. Food Science *48*,172.

MOTOKI, M., TORRES, J. A. and KAREL, M. 1982. Development and stability of intermediate moisture cheese analogs from isolated soybean proteins. J. Food Proc. Pres. *6*(1),41.

PAVEY, R. L. 1972. Controlling the amount of internal aqueous solution in intermediate moisture foods. Swift and Co., Research and Development Center, Oak Brook, Ill. Contract No. DAAG 17-70-C-0077, Food Laboratory, U.S. Army Natick Laboratories, MA 01760.

THERON, D. P. and PRIOR, B. A. 1980. Effect of water activity and temperature on *Staphylococcus aureus* growth and thermonuclease production in smoked snoek. J. Food Protection *43*,370.

TORRES, J. A. 1984. Exploration of the stability of intermediate moisture foods. Ph.D. thesis, Massachusetts Institute of Technology, Cambridge.

TORRES, J. A. and KAREL, M. 1984. Sorbic acid stability during processing and storage of an intermediate moisture cheese analog. J. Food Science (submitted for publication).

TORRES, J. A., MOTOKI, M. and KAREL, M. 1985a. Microbial stabilization of intermediate moisture food surfaces. I. Control of surface preservative concentration. J. Food Proc. Pres. *9*,00.

TORRES, J. A., BOUZAS, J. O. and KAREL, M. 1985b. Microbial stabilization of intermediate moisture food surfaces. II. Control of surface pH. J. Food Proc. Pres. *9*,00.

TROLLER, J. A. and CHRISTIAN, J. H. B. 1978. *Water activity and Food*, pp. 190, Academic Press, Inc., New York.

COEXTRUSION OF CARP (CYPRINUS CARPIO) AND RICE FLOUR

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ABSTRACT

Rice flour and varying amounts (10-35%) of deboned minced carp were coextruded resulting in a precooked blend that developed no detectable off-odors after being stored at room temperature for up to six months. In addition, the extrudates, along with nonextruded rice flour, were made into pakodas, a fried Indian snack food. Sensory triangle test data showed that up to 25% carp could be added before a statistical difference was noted. Hedonic sensory data demonstrated that consumer acceptable products relative to pakodas appearance, aroma, flavor, texture and overall acceptability can be made even at carp addition levels of up to 35%.

INTRODUCTION

Fish represents a food of good nutritional quality but because of its extreme perishability, a large portion of the world fish catch is lost. Therefore, fish preservation is an area of major concern. A major advantage of thermal food extrusion is that a room temperature shelf stable precooked product can be produced.

From a nutritional standpoint, the practice of blending foods is encouraged. Examples include the blending of various cereals and legumes.

In many parts of the world, carp *(Cyprinus carpio)* is a highly desirable species, and in these same areas, rice is usually the major cereal crop. The protein content of rice is relatively low (6-9%) while that of carp is significantly higher. To date, the authors are aware of only one investigation where fish has been coextruded (Murray *et al.* 1980). Defatted soy flour and minced Atlantic cod were successfully coextruded, but a Canadian taste panel found the resulting product to have an objectionable fishy flavor.

Therefore, the primary objective of this study was to evaluate the influence of extruded carp and rice flour ratios on the consumer acceptability of a popular Indian rice flour-based snack food (pakodas).

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MATERIALS AND METHODS

Ingredients

Commercial white rice flour was obtained (Comet Rice Mills, Inc., Houston, Texas).

Local lake carp averaging 3 kg each were netted and held live in fresh running water for 10 days to reduce muddy odor and taste. They were filleted, resulting in yields of 25-30% based on whole fish weight. The flesh was mechanically ground through a 0.63 cm hole plate using a household meat grinder. The minced flesh was combined and frozen at -36°C in 1 kg packages until used.

Proximate Analysis

Moisture, fat and protein were determined for the rice flour and minced carp (AOAC). A protein conversion factor of 5.95 was used for rice and 6.25 for fish. The same analyses were performed on the extrudates as well as for prepared pakodas with proportional protein conversion factors utilized.

Extrusion Formulations

Based on initial rice flour and carp moisture contents, a series of samples containing from no carp (control) to 10, 15 and 20% minced carp was made. The moisture content of the mixtures was adjusted to a total of 25% since preliminary extrusion runs indicated that products having moisture contents in excess of 30% were difficult to process. Potential protein denaturation was not considered. To increase the proportion of fish above 20%, the moisture content of the minced fish was reduced to 50% by baking 1 kg units at 120°C for 40 min. Using this cooked product, additions of 25, 30 and 35% carp to rice flour were made. Two kilogram flour/fish/water mixtures were blended in a 20 liter Model B20T Blackslee vertical mixer equipped with a wire ship. Mixing was for 5 min at speed 1, and 15 min at speed 2. A summary of all formulations used for extrusion are presented in Table 1.

Extrusion

A Model PL-V500 Brabender Plasticorder extruder equipped with a 3/1 screw and a 0.5 cm die was used. The unit was operated at 150 RPM with a barrel temperature of 150°C. Each of the seven formulations shown in Table 1 was extruded on three consecutive days in a sequential fashion for a total of 21 runs.

Table 1. Extrusion formulations*

Variable	% Rice Flour	% Carp/Form	% Added Water
1	100	0/raw	14
2	90	10/raw	7
3	85	15/raw	3
4	80	20/raw	0
5	75	25/cooked	4
6	70	30/cooked	2
7	65	35/cooked	0

* All formulations had calculated total moisture contents of 25% prior to extrusion.

Extrudate Drying, Grinding and Storage

The extrudates were collected and permitted to air dry for 24 h. Samples were ground in a Model 4 Wiley laboratory mill through a 1 mm opening screen. Representative samples of the ground products were placed in Mason jars, sealed, and stored at either -36°C or 22°C for up to six months for flavor evaluation. A sample of nonextruded rice flour was also subjected to these same storage conditions.

Pakodas Formulation

A standard recipe for pakodas, as shown in Table 2, was used. The ingredients were blended into a batter, 10 g units added to 210°C cooking oil, and fried for 2.5 min. The snacks were removed from the oil, drained on paper towels for one minute, and presented to the panel. The seven extruded products shown in Table 1, along with a nonextruded rice flour control, were formulated in pakodas.

Sensory Panel Makeup and Evaluation

A group of 22 Indian students and/or spouses familiar with pakodas attending Colorado State University was used as the panel. They included 15 males and 7 females, and ranged in age from 20 to 46.

Table 2. Pakodas recipe

100 g rice flour or ground extrudate
100 g water
2 g salt
0.5 g ground black pepper
40 g finely chopped onions
10 g finely chopped green Bell pepper

The same group was used to evaluate the odor properties of the eight samples placed in storage. Ground samples were presented at 0, 3, and 6 months of storage and the panel asked to describe any objectionable odors.

Freshly prepared pakodas were evaluated by the panel using standard procedures for the triangle test (Amerine *et al.* 1965). In addition, separate samples of each variable, including the nonextruded and extruded 100% rice flour controls, were evaluated for their appearance, aroma flavor, texture, and overall acceptability using standard procedures for a seven point hedonic scale (Amerine *et al.* 1965). Data were statistically evaluated using standard tables associated with each of these sensory tests (Amerine *et al.* 1965).

RESULTS AND DISCUSSION

Proximate Composition

As seen in Table 3, the rice flour used had an initial moisture content of 11.0% and a protein content of 6.9%, whereas raw carp had 81.0% moisture and 18.2% protein. Cooking carp decreased moisture, thereby, increasing protein and fat amounts. Both rice flour and carp were relatively low in fat.

All of the ground extrudates had a moisture content ranging from 7.0 to 7.5%, which would lead to good storage properties. Using the protein content of extruded rice flour as the base, it can be seen that even the incorporation of only 10% raw minced carp increased the amount of protein by 17%, and when 35% cooked minced carp was added, protein content increased 86%. Thus, these extrudates represent a source of precooked food of relatively high protein content that could be consumed as such or be incorporated into other foods. Even at the highest carp addition, the fat content was still only 2.32%.

Table 3. Proximate composition of ingredients, extrudates and Pakodas*

Product	% Moisture	% Protein	% Fat
Ingredients:			
Rice flour (RF)	11.0	6.9	0.42
Raw minced carp (RMC)	81.0	18.2	4.31
Cooked minced carp (CMC)	50.0	23.7	5.09
Extrudates:			
100% RF	7.4[a]	8.3[a]	0.47[a]
90% RF/10% RMC	7.2[a]	9.7[a]	0.92[a]
85% RF/15% RMC	7.4[a]	10.3[b]	1.14[b]
80% RF/20% RMC	7.5[a]	10.9[b]	1.44[b]
75% RF/25% CMC	7.3[a]	13.3[c]	1.80[c]
70% RF/30% CMC	7.0[a]	14.3[c]	2.06[c]
65% RF/35% CMC	7.1[a]	15.4[d]	2.32[c]
Pakodas:			
100% non-extruded RF	12.6[a]	6.4[a]	7.42[a]
100% extruded RF	12.2[a]	6.5[a]	7.17[a]
90% RF/10% RMC	12.5[a]	9.5[b]	7.15[a]
85% RF/15% RMC	12.9[a]	10.0[b]	7.15[a]
80% RF/20% RMC	13.1[a]	10.4[b]	7.11[a]
75% RF/25% CMC	14.6[b]	12.8[c]	7.03[a]
70% RF/30% CMC	15.2[c]	13.7[c]	7.00[a]
65% RF/35% CMC	16.0[c]	14.6[d]	7.00[a]

* All data represent the average of duplicate samples run on each of three separate products. Column data within the extrudates and Pakodas listings with different letters are significantly different ($p = .05$)

In looking at the pakodas composition, it can be seen that the frying process resulted in the absorption of some oil since fat contents for all products was in the neighborhood of 7%, which is still relatively low for a fried food. Factors such as frying temperature, time, and pakodas size would influence the amount of fat absorbed. Also, it can be seen that the extrusion process did not influence composition of the two 100% rice flour controls. It also appears that increasing the carp content influenced water retention, since the final moisture content of the pakodas increased with increasing carp addition and, as would be expected, protein content also increased. Using the 100% rice flour pakodas as controls, the addition of 10% carp resulted in a 48% increase in protein, while the highest carp addition (35%) resulted in pakodas containing 128% more protein than the traditional 100% rice product.

Extrudate Storage Stability

The odor properties of the seven extrudates shown in Table 3, along with those of the nonextruded rice flour control, were evaluated at 0, 3 and 6 months of storage at -36° and 22°C. The 16 samples were randomly presented in powdered form at room temperature to the 22 member sensory panel who were asked to describe any objectionable odor. Even after 6 months, no objectionable odor were reported in any of the samples. Thus, it appears that the extrusion process is well suited to the manufacture of a product that has good odor storage stability. Factors that accounted for good stability include the relatively low moisture and fat contents of the extrudates. In addition, no strong fishy odor, as was found by Murray *et al.* (1980), was apparent in this study, thereby, demonstrating that starting fish quality is of major importance. Possible explanations for this difference include differences in fish species used and extrusion conditions. The higher the extrusion temperature, the greater the potential to flash off volatile odors.

Sensory Triangle Tests

It should be remembered that the triangle test is soley a difference test and not a preference test. Freshly prepared pakodas were submitted to the 22 member Indian panel on three consecutive days with each day representing separate extrusion runs. These results are summarized in Table 4. As can be seen, the panel could not distinguish between pakodas made from traditional rice flour and extruded rice flour. When pakodas made from extruded rice flour and increasing amounts of carp were compared, the panel could not statistically distinguish between 10, 15, 20 and 25%

Table 4. Pakodas sensory triangle test results*

Comparisons	Number of Correct Responses**
100% non-extruded RF vs. 100% extruded RF	6
100% extruded RF vs. 90% RF/10% RMC	7
100% extruded RF vs. 85% RF/15% RMC	7
100% extruded RF vs. 80% RF/20% RMC	8
100% extruded RF vs. 75% RF/25% CMC	10
100% extruded RF vs. 70% RF/30% CMC	14
100% extruded RF vs. 65% RF/35% CMC	17

* Results are the average of three separate evaluations
** 12 correct responses required at p = .05

added carp. However, at carp levels of 30 and 35%, the panel could statistically make distinctions. Therefore, it can be concluded that at least 25% carp can be added to pakodas before major differences become apparent. In referring back to Table 3, it can be seen that pakodas made with 25% added carp have a protein content of 12.8%, which is 100% higher than in the 100% rice controls. The intensity and amount of spices and frying conditions can be significant factors in dictating the maximum amount of carp that can be added before detection is obvious.

Sensory Hedonic Tests

The hedonic test is a classical preference test and, as seen in Table 5, no sensory property of any product variable was judged to be disliked. Relative to pakodas appearance, no significant difference was apparent until at least 25% carp was added. However, products containing 35% carp were still rated as looking moderately good. The golden brown color that develops during frying appeared to minimize color differences. The aroma ratings were statistically comparable for pakodas containing up to 20% carp, while with flavor, significant differences were found at levels exceeding 15% addition. It is also interesting to note, with flavor, that both the 100% extruded rice flour control and the product containing 10% carp were statistically better than the 100% nonextruded rice flour control. Texture was the sensory attribute that was judged the lowest, even for the controls. However, the texture of pakodas containing 35% carp was still not objectionable. When the data for overall acceptability are considered, again all

Table 5. Pakodas sensory hedonic test results*

Product	Appearance	Aroma	Flavor	Texture	Overall Acceptability
100% non-extruded RF	6.4[a]	6.0[a]	6.2[b]	5.7[a]	6.3[a]
100% extruded RF	6.5[a]	6.0[a]	6.6[a]	5.6[a]	6.5[a]
90% RF/10% RMC	6.4[a]	6.2[a]	6.6[a]	5.6[a]	6.5[a]
85% RF/15% RMC	6.0[a]	6.0[a]	6.2[b]	5.3[b]	6.0[a]
80% RF/20% RMC	6.0[a]	5.7[b]	6.3[b]	5.2[b]	5.5[b]
75% RF/25% CMC	5.8[b]	5.5[b]	6.0[b]	5.0[b]	5.0[b]
70% RF/30% CMC	5.4[c]	4.6[c]	5.7[c]	4.5[c]	4.5[c]
65% RF/35% CMC	5.1[c]	4.6[c]	5.5[c]	4.2[c]	4.3[c]

*Results are the average of three separate evaluations. Scale used:
7. Like extremely; 6. Like very much; 5. Like moderately; 4. Neither like nor dislike; 3. Dislike moderately; 2. Dislike very much; 1. Dislike extremely;
Column data with different letters are significantly different ($p = .05$)

variables produced acceptable results but significant differences were apparent after 15% carp addition. Thus, the above data demonstrate that a extruded rice-based snack food containing up to 35% carp is acceptable to a native Indian population.

REFERENCES

AMERINE, M. A., PANGBORN, R. M. and ROESSLER, E. B. 1965. *Principles of Sensory Evaluation of Food.* Academic Press, New York.

AOAC Official Methods of Analysis. 1975. Washington, D.C.

MURRAY, B. P., STANLEY, D. W. and GILL, T. A. 1980. Improved utilization of fish protein — co-extrusion of mechanically deboned salted minced fish. Can. Inst. Food Sci. Technol. J. *13,* 125-130.

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HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

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