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Usefulness of the Stomacher in a Microbiological Regulatory Laboratory

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The relative efficiency of the Waring blender, the Stomacher 400, and the Stomacher 3500 for preparing food samples for microbiological analysis was studied. Comparative aerobic plate count (APC) values were determined on 671 samples, representing 30 categories of foods. Of the 26 categories of nonfatty foods, the blender gave significantly higher geometric mean APC values than those given by the Stomacher 400 and the Stomacher 3500 in 65 and 69% of the categories, respectively. In a comparison of the two stomacher models, the Stomacher 400 gave significantly higher geometric mean APC values than those given by the Stomacher 3500 in 73% of the food categories. Addition of Tween 80 to four categories of fatty foods at concentrations of 0.5, 1.0, and 2.0% did not raise the APC values given by either model of stomacher to the levels given by the Waring blender. Overall, the efficiency of both models of stomacher, relative to the blender and to each other, was specific and depended upon the particular food being analyzed.

The concept of stomaching as a means of sample preparation is relatively new, having been introduced by Sharpe and Jackson in 1972 (10). Stomaching was offered as a useful alternative to blending in preparing food samples for microbiological analysis. The design, operating principles, and advantages of the stomacher have been detailed by those authors and are not discussed here.

Three sizes of stomacher are commercially available (A. J. Seward and Co. Ltd., London, England) for handling sample volumes of 8 to 80 ml (Stomacher 80), 40 to 400 ml (Stomacher 400), and 300 to 3,000 ml (Stomacher 3500). The Stomacher 80, which is used in the microbiological analysis of cosmetic creams (3) and clinical specimens (7), would not be suitable for handling the larger sample sizes required in the microbiological examination of foods.

After the introduction of the stomaching concept, a number of papers evaluating the stomacher appeared in the foreign literature. Tuttlebee (11) reported that the Stomacher 400 gave significantly higher aerobic plate count (APC) values than those given by homogenization with the Ato-mix blender or with a mortar and pestle in a majority of 89 samples comprising five food categories. Baumgart (4) compared total counts in 10 categories of meats prepared by the Stomacher 400 and the Ultra-Turrax homogenizer and found no significant differences. Kihlberg (6) reported comparable plate counts in minced meat, veal brawn, and frozen fish fillets prepared by the Stomacher 400 and the Ultra-Turrax homogenizer.

Since the Waring blender is the type of homogenizer probably most widely used in this country for preparing foods for microbiological analysis, any evaluation of the stomacher should include a high-speed blender as the standard method of homogenization. Numbers and types of food categories should also be considered in any evaluation of the stomacher. In performing its regulatory role, the typical microbiological laboratory of the Food and Drug Administration analyzes a rather wide variety of foods, and any comparative evaluation should include an expanded list of foods, particularly those which are most apt to be examined routinely. Since the Stomacher 3500 has not yet been evaluated and since this model would be the one most often used in analyzing larger food samples, this model, along with the model 400, should be included in the evaluation. The results of a study encompassing the three factors stated above are reported here.

MATERIALS AND METHODS

Sample collection and handling. With the exception of oysters, all food samples were obtained from local retail outlets in the Washington, D.C., or Minneapolis metropolitan areas. Samples of oysters were

harvested from shellfish beds in Mobile Bay, Ala., by the Gulf Coast Technical Services Unit, Dauphin Island, Ala. The shellstock was shucked by standard aseptic techniques (1) and shipped air express to the Washington, D.C., laboratory for analysis. For all food categories, each sample consisted of 200 g of material. A total of 671 samples, representing 30 food categories, was examined in this study.

Samples were screened to remove any sharp objects that might puncture the stomacher bags (e.g., shell fragments in nut meats, seeds in pears and apples, and shell bits in oysters). Foods with sharp or protruding edges, such as macaroni and rice, were allowed to soften by presoaking for 30 min in diluent. Each 200-g sample was subdivided into three portions: (i) 50 g for the blender, (ii) 50 g for the Stomacher 400, and (iii) 100 g for the Stomacher 3500.

Blending. Two hundred milliliters of Butterfield phosphate buffer (5) was added to the 50-g sample portion in the blender jar and blended for 2 min at high speed (14,000 rpm). For uniformity, samples that were soaked before stomaching were also soaked before blending.

Stomaching. Both the intermediate-sized Stomacher 400 and the large Stomacher 3500 were used in the evaluation. Before initiation of the study, a representative of the local distributor of the stomachers (Cooke Laboratories, Alexandria, Va.) was asked to examine the stomacher paddles and adjust the paddles to the optimal operating position if necessary. For the Stomacher 400, 200 ml of Butterfield phosphate buffer was added as the diluent to the 50-g sample contained in a sterile 18- by 30-cm bag. For the Stomacher 3500, 900 ml of diluent was added to the 100-g sample portion in a sterile 38- by 51-cm plastic bag. Each sample portion was stomached for 2 min.

Tween 80 was added to foods with a relatively high fat content (ground beef, pork sausage, butter, and cheese) to give a final concentration of 0.5, 1.0, and 2.0%. A control containing no surfactant was included.

Counting procedures. Dilutions in Butterfield phosphate buffer were made from the blender jar or stomacher bag. For each dilution, inocula were plated in triplicate by using standard plate count agar. Plates were incubated at 35°C for 48 ± 2 h, then counted manually with a Quebec colony counter according to procedures outlined by the Association of Official Analytical Chemists (2).

Statistical analysis. Significant differences in APC values obtained by blending and stomaching were determined by using the formula

$$Z = \frac{M_1 - M_2}{\sqrt{\frac{V(Y_{1i})}{(n_1)(x_1)^2} + \frac{V(Y_{2j})}{(n_2)(x_2)^2}}}$$

TABLE 1. *Geometric means of APC values of nonfatty foods prepared by blending and stomaching*

Food type	No. of samples	APC/g (geometric mean)				
		Blender value	Stomacher 400		Stomacher 3500	
			Value	%[a]	Value	%[a]
Apples[b]	22	1.2×10^4	5.8×10^3	48	9.6×10^3	80
Figs	21	2.1×10^3	5.4×10^2	26	4.0×10^2	19
Pears[b]	26	4.2×10^3	2.3×10^3	55	1.5×10^3	36
Raisins	24	2.6×10^2	3.5×10^2	135	2.9×10^2	112
Broccoli[b]	22	2.3×10^6	3.1×10^6	135	3.0×10^6	130
Cabbage[b]	20	1.4×10^6	8.7×10^5	62	6.3×10^5	45
Carrots[b]	23	9.6×10^6	8.9×10^6	93	8.1×10^6	84
Cauliflower[b]	20	1.3×10^6	1.2×10^6	92	1.7×10^6	131
Mushrooms[b]	20	1.4×10^9	1.7×10^9	121	1.3×10^9	93
Okra[b]	21	7.6×10^7	8.3×10^7	109	5.4×10^7	71
Pecans	20	6.3×10^2	7.1×10^2	113	1.4×10^4	2,220
Walnuts	20	3.7×10^3	2.1×10^3	57	1.2×10^3	32
Pepper	20	1.4×10^7	2.1×10^7	150	1.7×10^7	121
Thyme	22	3.6×10^4	2.8×10^4	78	2.1×10^4	58
Oysters	28	4.0×10^4	4.5×10^4	113	3.7×10^4	93
French fries[c]	32	5.9×10^2	5.0×10^2	85	1.8×10^3	305
Onion rings[c]	22	4.9×10^3	4.1×10^3	84	2.0×10^3	41
Macaroni	22	1.4×10^4	1.1×10^4	79	8.4×10^3	60
Rice	35	1.5×10^4	1.5×10^4	100	1.6×10^4	107
Dough	24	2.6×10^4	1.5×10^4	58	1.6×10^4	62
Cake	23	1.2×10^3	7.4×10^2	62	5.4×10^2	45
Tuna pot pie	25	1.6×10^3	1.1×10^3	69	9.9×10^2	62
Meat extender	24	1.2×10^3	3.6×10^2	30	2.2×10^2	18
Protein supplement	23	1.2×10^4	1.9×10^4	158	2.5×10^4	208
Chocolate	20	4.8×10^4	2.1×10^4	44	7.8×10^3	16
Carmine dye	15	6.8×10^5	1.8×10^5	27	1.2×10^5	18

[a] Expressed as percentage of blender values.
[b] Fresh produce.
[c] Breaded.

TABLE 2. *Statistical analysis of paired APC values of nonfatty foods prepared by blending and stomaching*

Food type	No. of samples	% of samples								
		Blender vs. Stomacher 400			Blender vs Stomacher 3500			Stomacher 400 vs Stomacher 3500		
		BL[a,b] > 400[a]	BL[b] < 400	NS[c]	BL > 3500[a]	BL < 3500	NS	400 > 3500	400 < 3500	NS
Apples[d]	22	46[e]	41	14	46	41	14	36	55	9
Figs	21	81	5	14	76	10	14	52	19	29
Pears[d]	26	77	19	4	65	19	15	65	31	4
Raisins	24	17	46	38	17	25	58	21	13	67
Broccoli[d]	22	50	50	0	36	59	5	27	41	32
Cabbage[d]	20	55	35	10	55	35	10	60	40	0
Carrots[d]	23	35	26	39	44	22	35	44	30	26
Cauliflower[d]	20	50	45	5	35	45	20	45	50	5
Mushrooms[d]	20	30	50	20	50	50	0	50	25	25
Okra[d]	21	29	48	24	62	29	10	57	24	19
Pecans	20	40	45	15	15	85	0	0	100	0
Walnuts	20	55	30	15	55	30	15	55	30	15
Pepper	20	10	25	65	50	15	35	60	10	30
Thyme	22	64	23	14	77	14	9	64	23	14
Oysters	28	32	43	25	39	29	32	36	29	36
French fries[f]	32	34	22	44	41	50	9	31	59	9
Onion rings[f]	22	41	14	46	82	5	14	59	18	23
Macaroni	22	32	45	23	64	23	14	54	14	32
Rice	35	14	11	74	11	17	71	14	23	63
Dough	24	38	25	38	38	25	38	13	38	50
Cake	23	65	13	22	70	9	22	61	9	30
Tuna pot pie	25	84	4	12	96	4	0	32	4	64
Meat extender	24	71	17	13	75	13	13	42	8	50
Protein supplement	23	30	52	17	39	48	13	39	57	4
Chocolate	20	95	0	5	100	0	0	100	0	0
Carmine dye	15	100	0	0	100	0	0	60	0	40

[a] BL, Waring blender; 400, Stomacher 400; 3500, Stomacher 3500.
[b] Significant difference at the 95% confidence level.
[c] NS, No significant difference at the 95% confidence level.
[d] Fresh produce.
[e] Because percentages are expressed as whole numbers, totals in certain instances will be 99 or 101%.
[f] Breaded.

where M_1 and M_2 are APC values of paired samples, $V(Y_{1i})$ and $V(Y_{2j})$ are the variances of the plate count values appropriate to M_1 and M_2, n_1 and n_2 represent the number of plates per dilution, and $(x_1)^2$ and $(x_2)^2$ are the dilution ratios squared. Z is the computed standard normal deviate, and differences in paired APC values are significantly different at the 95% level for Z values of ± 1.96.

RESULTS AND DISCUSSION

Table 1 shows a comparison of APC values obtained in 26 categories of nonfatty foods prepared by the blender, the Stomacher 400, and the Stomacher 3500. The selection of these foods was based, in part, on the probability of their being encountered in a routine analytical situation. An attempt was also made to include foods that would be representative of the basic food groups (fruits, vegetables, nut meats, condiments, and shellfish) as well as a wide variety of miscellaneous foods which may have regulatory significance.

The blender gave significantly higher percentages of APC values (geometric means) than did the Stomacher 400 in 17 (65%) of the 26 categories, whereas the Stomacher 400 gave higher APC values in 8 (31%) of the food categories. In one food category, rice, the geometric means obtained by the two procedures were identical. In a comparison of the blender with the Stomacher 3500, the blender gave significantly higher geometric mean counts in 18 (69%) of the 26 categories, and the Stomacher 3500 gave higher mean counts in 8 (31%) of the categories. In a comparison of the two stomacher models, higher mean counts were obtained with the Stomacher 400 in 19 (73%) of the categories and with the Stomacher 3500 in 7 (27%) of the categories.

The developers (10) state that the pounding of the sample by the steel paddles removes bacteria from the food particles partly by violent shearing forces as the liquid moves from side to side and partly by the actual compression of the sample itself as it becomes trapped under the

stomacher paddles. Because of the much larger volumes accommodated by the Stomacher 3500, the sample bag is shielded by a rubber apron from direct contact with the metal paddles that hit the bag in the Stomacher 400. This tempered force with which the paddles hit the stomacher bag in the model 3500 is probably responsible for the lower level of bacteria removed by this model.

A statistical analysis of the three methods of paired sample preparations is shown in Table 2. Making generalizations from the data in this table would be difficult. For example, of the four categories of fruits used in the evaluation, both stomacher models performed better than the blender with the raisin samples. The blender, however, performed better than both stomachers with the apple, fig, and pear samples. The only reasonable conclusion, therefore, seems to be that the relative efficiency of the blender and the stomachers depended on the specific food being analyzed.

In their original paper, Sharpe and Jackson (10) acknowledged that the stomacher gave lower counts than those given by the Ato-mix blender only in those samples of high fat content, e.g., fatty beef cuts, short crust pastry, and dairy cream. In a follow-up paper, Sharpe and Harshman (9) reported that a 1% concentration of Tween 80 added to fatty foods would restore the recovery efficiency of the stomacher. In the evaluation reported here (Table 3), three concentrations of Tween 80, in addition to a control with no surfactant, were added to four categories of fatty foods. No concentration of Tween 80 added to any of the four food categories of fatty foods raised the APC values given by either stomacher model to those given by the blender. Moreover, addition of Tween 80 had no significant effect on the bacteriological counts of food samples whether prepared by blending or stomaching. The stomaching of the butter samples and, to a lesser extent, the pork sausage samples presented a special problem, because these samples tended to adhere to the bag. At the very onset of stomaching a thick coating formed on the interior of the bag that no amount or degree of pounding could dislodge.

The data show that the efficiency of the stomachers varies according to the type of food being examined. In regulatory laboratories such as those of the Food and Drug Administration, a wide variety of foods is routinely examined. Even though 30 food categories were used in this evaluation, the food-specific efficiency of the stomacher would require extrapolation from these data in determining whether to use the stomacher with other foods. Such a practice would be undesirable from both a microbiological and a regulatory standpoint. These conclusions should not be misinterpreted as being a complete refutation of the stomaching concept. In certain food categories stomaching indeed gave counts comparable to those obtained by blending, and the stomacher could be most advantageously used in quality control laboratories analyzing large volumes of those foods.

After the preparation of this manuscript, Schiemann (8) reported an evaluation of the Stomacher 400 in preparing three types of food

TABLE 3. *Comparison of APC values of fatty foods prepared by blending and stomaching with various concentrations of Tween 80*

Food type	No. of samples	Concn of Tween 80 (%)	APC/g (geometric mean)				
			Blender value	Stomacher 400		Stomacher 3500	
				Value	%[a]	Value	%[a]
Ground beef	18	0	5.2×10^6	4.9×10^6	94	4.1×10^6	79
		0.5	6.3×10^6	5.7×10^6	91	4.6×10^6	73
		1.0	7.1×10^6	6.1×10^6	86	5.1×10^6	72
		2.0	7.3×10^6	5.9×10^6	81	5.0×10^6	69
Pork sausage	19	0	3.2×10^7	2.1×10^7	66	1.9×10^7	59
		0.5	3.6×10^7	2.4×10^7	67	2.0×10^7	56
		1.0	3.8×10^7	2.5×10^7	66	1.8×10^7	47
		2.0	4.3×10^7	2.2×10^7	51	2.0×10^7	47
Cheese	20	0	2.1×10^5	2.2×10^5	105	1.3×10^5	62
		0.5	2.5×10^5	2.0×10^5	80	1.4×10^5	56
		1.0	2.2×10^5	1.8×10^5	82	1.2×10^5	55
		2.0	2.3×10^5	1.7×10^5	74	1.2×10^5	52
Butter	20	0	8.4×10^4	2.0×10^4	24	1.0×10^4	12
		0.5	7.5×10^4	2.4×10^4	32	1.4×10^4	19
		1.0	8.2×10^4	3.1×10^4	38	1.7×10^4	21
		2.0	5.0×10^4	3.4×10^4	68	1.2×10^4	24

[a] Expressed as percentage of blender values.

samples for microbiological analysis. In general, no significant difference in APC values was observed when these samples were prepared by blending and by stomaching. The somewhat different results reported in our paper should further emphasize the food-specific efficiency of the stomacher and the absolute need for initially using the stomacher on a comparative trial basis before incorporating its use into any routine analytical procedure.

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