A NOTE ON A RAPID METHOD FOR THE DETERMINATION OF LIPIDS IN BREWING ADJUNCT CEREALS[1]

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This note presents some studies on a rapid and reproducible method for the determination of lipids in brewing adjunct cereals. The study is of particular interest because of the extensive collaborative work done in recent years in search of an improved lipid extraction procedure (1,2).

The usual method for the determination of lipids in brewing adjunct cereals involves a 6-hour Soxhlet extraction of the ground sample with low-boiling petroleum ether (30°–60°C.), followed by the evaporation of the solvent and drying of the extracted liquids under standardized conditions (3). In 1959, the Subcommittee on Cereal Adjunct Analysis of the American Society of Brewing Chemists recommended that the extraction time be reduced to 2 hours, and this recommendation has been included in the official methods of the Society (4). This shorter method, while it is an obvious improvement, still requires the use of ground samples and takes approximately 4 hours to complete.

West and Lautenbach (6) recommended the use of a simple column for the extraction employing low-boiling petroleum ether or diethyl ether as the extractant. This procedure simplified the apparatus and simultaneously reduced the extraction time to 3 hours. It retained the disadvantage of requiring a preground sample and, in addition, suffered from the difficulty of using relatively large volumes of flammable extractant.

The Subcommittee on Cereal Adjunct Analysis attempted to use a modified Waring Blendor, in which the sample was extracted by grinding with solvent twice for 2 and 3 minutes, respectively (1). It was found that the range of values obtained for any given sample was rather large. For one sample of corn grits for which the mean value of lipid content was 1.01%, the range in one case was ± 0.28% and in the duplicate set was ± 0.43%.

The method reported here reduces the extraction time to 2.5 minutes without pregrinding and employs the Servall Omnimixer.[3]

A 10-g. unground sample is placed in the metal chamber of the Omnimixer, covered with redistilled low-boiling (30°–60°C.) petro-

[1] Manuscript received April 4, 1960. Contribution from Liebmann Breweries, Brooklyn, New York.
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leum ether (75–100 ml.), and extracted by grinding for 2.5 minutes at a potential of 90 volts. During grinding, the cup is immersed in a cold-water bath. The extract is gravity-filtered into a tared receiving flask, and after the residue has been washed onto the filter with small portions of petroleum ether, the solvent is evaporated from the open flask on a hot-water bath. The extracted oils are dried at 103°–105°C. for 75 minutes, cooled to room temperature in a desiccator over calcium chloride, and weighed to the nearest mg.

Complete extraction was routinely effected within 2.5 minutes, and in some cases within 1 minute.

Several samples of adjuncts were analyzed and excellent replication was obtained (Table I).

TABLE I

DETERMINATION OF OILS IN REPLICATE SAMPLES OF ADJUNCT CEREALS BY THE 2.5-MINUTE OMNIMIXER EXTRACTION PROCEDURE

ADJUNCT	NUMBER OF REPLICATE SAMPLES	OIL (Mean Value)	RANGE
		%	%
Rice	4	0.70	± 0.03
Corn grits	3	0.51	± 0.01
Corn flakes A	3	0.53	± 0.01
Corn flakes B	3	0.59	± 0.02

The sample of corn flakes, B, was also assayed in triplicate by the 6-hour Soxhlet extraction method. The mean value found was 0.57% oil, and the range was ± 0.06%.

Drying time of the extracted oils was standardized at 75 minutes. Prolonged drying gave erratic results. Seven 10-g. subsamples of corn flakes and rice were extracted in the Omnimixer and dried for

TABLE II

RATE OF DRYING OF OILS FROM RICE AND CORN FLAKES IN AN ALCONAP AIR-CONVECTION OVEN AT 104°C. AND IODINE UPTAKE BY THE DRIED OILS

DRYING TIME	RICE		CORN FLAKES	
	Oil Extracted	Iodine Absorbed [a]	Oil Extracted	Iodine Absorbed [a]
minutes	*g*		*g*	
0	. .	0.63	. . . .	0.52
30	0.120	0.63	0.099	0.52
45	0.101	0.63	0.097	0.52
60	0.085	0.64	0.062	0.51
75	0.082	0.63	0.062	0.51
90	0.087	0.44	0.055	0.31
120	0.098	0.36	0.064	0.12

[a] Iodine uptake is expressed in milli-equivalents per 10 g. of original sample.

various periods of time at 104°C. in an Alconap air-convection oven. The residual oils were weighed after cooling in a desiccator over calcium chloride for 20 minutes. The mass of the residual oil decreased for approximately the first 75 to 90 minutes of drying time and then started increasing (Table II).

The extent of oxidation of the extracted oils was determined by iodine uptake (8). Concomitantly with the mass increase of the extracted oil, the iodine uptake decreased. Even in the case of corn oil where there was a further decrease in the mass of the oil from 75 to 90 minutes, there was already a decided decrease in the iodine uptake after 75 minutes. This indicates that after 75 minutes' drying, some oxidation occurs which brings about an increase in weight.

Moisture did not interfere in the drying experiment. Several freshly extracted oils were analyzed for moisture by the Karl Fischer method (5), and no water was present.

The mass loss during the first 75 minutes of drying time is, apparently, a loss of residual solvent and, possibly, of some other volatile substances. While solvent is present, the temperature of the sample is relatively low. As the sample temperature increases, oxidation sets in.

The Omnimixer and the 6-hour Soxhlet extraction procedure were compared for rice, corn flakes, and corn grits. The Wilcoxon matched-pairs, signed-ranks test (7) was applied to the results to determine whether a difference could be demonstrated between the two methods at the 1%-significance level. The results are shown in Table III.

Wilcoxon showed that the T-statistic, a rank measure, must be equal to or numerically smaller than a tabulated critical value in order to demonstrate a significant difference between two sets of results at any specified level of significance. The magnitude of the critical T-statistic depends upon the number of sample pairs (n).

TABLE III

A STATISTICAL EVALUATION OF THE RESULTS OBTAINED BY THE 2.5-MINUTE OMNIMIXER EXTRACTION AND THE 6-HOUR SOXHLET EXTRACTION OF CEREAL ADJUNCTS BY THE WILCOXON PROCEDURE

CEREAL	NUMBER OF SAMPLES	WILCOXON T-VALUE	
		Calculated	Critical Value[a]
Rice	22	70.5	49
Corn flakes	9	12.5	2
Corn grits	8	14.0	0

[a] Critical value at 1% level of significance.

From Table III, the computed T-statistic is greater than the critical T-statistic, and, therefore, no difference between the two methods was demonstrated at the 1% level of significance.

Some comparative determinations on corn and rice by the Omnimixer method, the 6-hour Soxhlet extraction procedure, and the method of West and Lautenbach (6) are shown in Table IV.

TABLE IV

COMPARISON OF RESULTS FOR OIL CONTENT OF CORN AND RICE SAMPLES IN THREE PROCEDURES

SAMPLE	OILS		
	Omnimixer [a]	Column	Soxhlet [b]
	%	%	%
Corn 1	0.57	0.59	0.61
2	0.60	0.60	0.57
3	0.60	0.60	0.55
4	0.51	0.45	0.46
5	0.71	0.66	0.64
Rice 1	0.93	1.02	1.00
2	0.65	0.63	0.66
3	0.96	0.90	0.96
4	0.84	0.77	0.82
Wilcoxon-T [c]		8.5	12.5
Critical-T		2.0	2.0

[a] Extraction for 2.5 minutes.
[b] Extraction for 6 hours.
[c] T-values determined by comparing the Omnimixer method with each of the other methods.

The Wilcoxon test (7) failed to demonstrate a difference between the results obtained by the three methods at the 1%-significance level.

With the Omnimixer method, the use of diethyl ether was compared with that of petroleum ether as an extractant for both rice and corn. On application of the Wilcoxon test (7) to the results of an experiment in which seven samples were used, no difference could be found in the results with either extractant at the 1%-significance level.

Acknowledgment

The technical assistance of Peter Liebmann, Alex Kuvish, and Sam Vento is gratefully acknowledged. Thanks are also due to John Bain for the statistical work.

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