THE MICROFLORA OF MILLED WHEAT PRODUCTS

by Kenneth Neil Smith, B.Sc.,
University of Manitoba.

A Major Thesis submitted to the
Graduate Studies Committee of
The University of Manitoba
in candidacy for the
Degree Master of Science.
1948

ACKNOWLEDGMENTS

The writer is pleased to express appreciation to:

Dr. N. James, Department of Bacteriology and Animal Pathology, for his liberal advice and assistance during the course of the investigations reported and in the preparation of the manuscript.

Dr. C. H. Goulden, Officer-in-Charge, Dominion Laboratory of Cereal Breeding, Winnipeg, and Honorary Lecturer in Statistics, The University of Manitoba, for his advice concerning the statistical portion of the work.

Dr. J. E. Machacek, Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, for identifying the species of Penicillium.

Mr. J. A. Hessel, Chemist, The Ogilvie Flour Mills Company Limited, for supplying the samples of flour used in the main investigations.

Dr. J. A. Anderson, Chemist-in-Charge, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, for his cooperation in providing the milled products used in the preliminary studies.

TABLE OF CONTENTS

INTRODUCTION

Wheat flour is the basic food for a large part of the world's population. In 1946 the milling industry in Canada produced 14,984,287 barrels of flour for export alone. This was valued at $126,733,077 (16). Consequently, any factor that affects the quality of Canadian flour is of economic importance to those concerned with this important industry and is worthy of serious study. Relatively little is known about the microflora of Canadian flour although it might be expected that the flora of the product would affect fermentation in the bread-making process and be responsible for certain characteristic flavours in the finished product. A few defects have been studied in some detail elsewhere, although the information available at the present time must be accepted as fragmentary. Certain bacteria in flour have been found to produce sourness, others rancidity and still other bacteria ropiness in bread. Mustiness has been attributed to molds and actinomycetes. It might be expected that flour would contain a variety of soil and dust types of organism that could cause these defects under certain conditions of manufacture. It would appear logical therefore that fundamental information on the flora of normal flour should be available as a background for studies relating to specific problems that confront the miller from time to time. Accordingly this study was undertaken to provide data on the bacteria, yeasts and fungi in certain brands of flour milled commercially in Winnipeg.

HISTORICAL

Most investigations on the microbiology of flour have been undertaken to determine the part played by micro-organisms in flours with certain defects. Consequently, only little information is available on the microflora of normal flour. Tanner (15), 1944, outlined the history of the microbiology of flour. This outline contains the following information: Hoffman, 1896, evidently was the first to plate flour. Arnoldow, 1908, found that most bacteria and molds grew in flour when the moisture content was over 17 per cent. Kühl, 1911, isolated butyric acid bacteria from abnormal flour and reported finding B. subtilis on all flours investigated. McHargue, 1920, Street, 1922, and other earlier investigators reported that bacteria and molds did not grow in flour when the moisture content was less than 13 per cent. Turley, 1922, reported bacterial counts on flour which varied between 310,000 and 5,200,000 per gram. Satory and Satory, 1926, isolated A. fumigatus from flour with a musty odor.

Tanner, (15), 1944, also comprehensively reviewed the history of the problem of rope. This review shows that investigators found organisms of the B. mesentericus group (present in flour) responsible for rope in bread and that this condition could be controlled by adopting proper preventative measures.

Kent-Jones and Amos, (11), 1930, introduced the practice of adding sand to the initial dilution. This gave a higher

count than when no sand was added. A procedure for counting rope spores in flour was also worked out by them. They found that the washing of grain preparatory to milling caused a substantial reduction in numbers of micro-organisms on grain. They reported that freshly milled patent flour contained from 2,000 to 52,000 bacteria per gram, with an average number of 20,000. Straight run flours contained from 5,000 to 157,000 bacteria per gram, with an average number of 50,000, while lower grades gave counts ranging as high as 300,000 bacteria per gram. These counts were obtained on nutrient agar at 37 degrees C. (called "blood heat" organisms). They found such a great variation in numbers of organisms growing at 25 degrees C. (called "cool" organisms) that no attempt was made to set up limits. In addition they reported that the number of micro-organisms in flour decreased during storage -- the "blood heat" organisms decreasing more rapidly than the "cool" organisms. The decrease depended on the moisture content of the flour and on the temperature and humidity during storage. Humidity influenced the decrease more than temperature. These investigators isolated E. coli from flour, claiming that it originated in the water used for washing the wheat. B.subtilis and members of the B. mesentericus group (rope organisms) were isolated from nearly all of the samples examined. Amos (13), 1939, isolated M. ureae and M. liquifaciens and a species of Flavobacterium (a yellow rod considered to be epiphytic on

wheat) from flour. He found a species of Streptococcus and a torula on most samples.

Gustafson and Parfitt (7), 1933, studied the effect of the total numbers of bacteria present on the development of rancidity in soft wheat flours. Using the technique of Kent-Jones and Amos these investigators found that best grade patent flours contained from 9,000 to 146,000 bacteria per gram, and poorer grades from 30,000 to 180,000 bacteria per gram. It was also found that experimentally milled samples gave a higher count than commercially milled flours. They reported that the total bacterial count tended to decrease during storage and that the bacterial count was in no way correlated with the development of rancidity during storage.

Holtman (8), 1935, also investigating the problem of rancid soft wheat flour, found that bacteria decreased in numbers through the different stages of milling. He reported that freshly milled normal flour contained on the average 23,000 bacteria and 1800 molds per gram. These counts increased during storage under favorable conditions of moisture and temperature. Overbleaching of the flour tended to increase the microbial oxidation of the flour. He isolated B. mesentericus, a species of Flavobacterium and a species of Sarcina from the flour examined.

Barton-Wright (1), 1938, sought to find if during storage the bacterial and fungal flora showed any marked changes in nature and number which could be correlated with chemical

changes that have been observed. He modified the technique of Kent-Jones and Amos by using a synthetic medium. He reported an initial count of 40,000 bacteria per gram for low grade flours and also stated that bacteria decreased during storage. Fungi increased to a maximum during storage when there was a moisture content of 16 per cent or over and a favorable temperature. Ninety per cent of the molds belonged to the genus Penicillium. He attributed mustiness in flour to fungi and not to bacteria.

The same author with Tomkins (2), 1940, studied the relationship of moisture content and temperature to the growth of molds in flour. They found that the critical water content for flour at which mold growth could take place was 16 per cent when there was a storage relative humidity of 79 per cent and a temperature of 20 degrees C. The critical water content increased as the temperature decreased.

Christensen (6), 1946, investigated the various factors that influence the number of molds cultured from a sample of flour. He found that the count was influenced by the method of making the dilution, the medium used for plating, and the method of counting the colonies.

PRELIMINARY STUDIES

I. Experimentally Milled Samples

In order to provide information on the flora of various milled wheat products, two samples of unwashed wheat were milled, at different dates, in the Research Laboratory of The Board of Grain Commissioners for Canada, in Winnipeg. Counts were made on each sample and on - 1. bran, 2. bran chips, 3. coarse shorts, 4. fine shorts, 5. feed flour, 6. first clear flour, 7. first patent flour and 8. second patent flour.

Each sample was shaken twenty-five times by hand. Observing aseptic technique a 10 gram portion was weighed on a balance accurate to 0.01 of a gram. This was accomplished by using small pyrex beakers that were flamed and cooled previous to use. The 10 gram portion was then transferred to a 6 ounce screw top bottle containing 93 ml. of sterile water (sufficient to bring the flour and water to 100 ml. or a dilution of 0.1) and a small quantity of fine sterile gravel. This dilution was shaken on a mechanical shaker for 30 minutes. Afterwards the flour was allowed to settle for approximately 10 minutes before 10 ml. of the supernatant liquid were pipetted off to make the second dilution. This dilution and all remaining dilutions were shaken 25 times by hand immediately before transfers were made. Nutrient agar was the medium used for bacteria and Czapek's agar (acidified just before pouring with

0.5 ml. of 10% aqueous lactic acid per 100 ml. of medium) was the medium used for fungi and yeasts. Incubation was at 25 degrees C. for 6 days.

The microbial content of the different milled products is shown in Table 1. The numbers represent the average count obtained on duplicate plates from 10 replications of each sample. The raw counts are presented in Appendix A. Section I.

Table 1. Average numbers of bacteria, fungi and yeasts per gram in experimentally milled wheat products.*

Product	Bacteria		Fungi		Yeast	
	Smpl. 1.	Smpl. 2.	Smpl. 1.	Smpl. 2.	Smpl. 1.	Smpl. 2.
Wheat	559000	699000	146	355	9170	11255
Bran	502000	247000	61	187	6510	2966
Bran chips	422000	369000	144	1255	2920	2000
Coarse shorts	301000	321000	104	1183	2500	1024
Fine shorts	407000	199000	101	264	3020	728
Feed flour	223000	66500	43	101	1700	372
1st clear	47000	20800	31	44	530	235
1st patent	13000	2950	17	16	260	128
2nd patent	12000	4630	14	34	490	95

* Each figure is based on counts from 20 plates.

The number of bacteria found in a product was exceedingly large when compared with the number of fungi and yeast in the same product. All products contained more yeasts than fungi. There was a definite but irregular decrease in the microbial content of the milled products from the bran to the second patent flour. The highest counts were obtained from those products which consisted chiefly of the seed coat of the wheat, e.g. bran and bran chips. A significantly lower count was obtained from the clear and patent flours. The patent flours harboured lower numbers than the clear flour. This would indicate that most of the organisms are contained in the seed coat - the less bran a product contains, and the more highly refined it is, the lower the content of micro-organisms.

Replications in a number of instances did not agree closely. A few gave such high counts of fungi as to greatly influence the average count of the product.

II. Sampling Procedure

The disagreement of counts between some replications of samples encountered in the foregoing study prompted an investigation on the sampling procedure. Counts were made on fungi only. It has been shown that the variation in fungal counts between replicate plates conforms to expectancy on the basis of random sampling from a biological population (9). Therefore this investigation was designed to find if the effect of the variation between replicate dilutions or the variation

between replicate 10 gram portions, or the variation inherent in the sample was responsible for the disagreements in counts between replicate samples.

An approximate 100 gram sample was removed at random on three successive days from one large sample of clear run flour. Then, observing aseptic technique the sample was thoroughly mixed on a large sheet of sterile paper by following a standard procedure. Four replicate 10 gram portions were weighed from each 100 gram sample and diluted to 0.1 by following the procedure used in the previous study. The four replicate 0.1 dilutions were shaken for thirty minutes on a mechanical shaker. Afterwards each dilution was shaken an additional twenty-five times by hand and agitated continuously to keep the flour in suspension while a 10 ml. aliquot was pipetted to each of four 90 ml. sterile water blanks. This provided four replicate 0.01 dilutions from each 0.1 dilution. Each of the 0.01 dilutions was shaken twenty-five times by hand and continuously agitated while a 1 ml. aliquot was pipetted to each of five plates. The medium used was acidified Czapek's agar. Incubation was at 25 degrees C. for six days.

The counts obtained were analyzed for significant differences between (a) replicate dilutions, (b) replicate 10 gram portions of a sample and (c) replicate samples by carrying out an analysis of variance. The results are shown in Table 2. The raw counts and the data necessary for an analysis of variance may be found in Appendix A. Section II.

Table 2. Effects of sampling procedure on the count of fungi in flour.

Source of variation	Degrees freedom	Mean square	F. value	F.at 5%
Dilutions	36	8.91	.73	1.45
10 gram portions	9	3.47	.31	1.92
Samples	2	196.91	16.23	3.04
Error	192	12.13		

It may be observed from the above analysis that neither the variation between replicate dilutions from one portion, nor the variation between replicate 10 gram portions of a sample significantly affected the variation in fungal counts. From this it would appear that the procedure followed in weighing out a 10 gram portion of a sample and in making dilutions from it was satisfactory. The results, however, show a highly significant difference between replicate samples. This would indicate that the variation in the number of fungi cultured from replicate samples was inherent in the samples. Therefore, to obtain a true picture of the number of fungi in flour it would be necessary to plate replicate samples.

III. Methods and Media.

An investigation was carried out to determine if the method of treating the initial dilution had any significant effect on the number of fungi cultured from a sample of flour. Also in this experiment two media, acidified Czapek's agar and Christensen's malt-salt agar (6), were compared to determine if they differed significantly in their effect on the number of fungi cultured from a sample of flour.

Ten different flour samples were used in this investigation. A 10 gram portion of each sample was weighed and diluted to 0.1 by following the procedure used in the previous experiments. The 0.1 dilution was shaken for two minutes on a mechanical shaker. Immediately afterwards, while the flour was still in suspension, a 10 ml. aliquot was pipetted off and used to make a 0.01 dilution. The initial dilution was then shaken an additional twenty-eight minutes on the mechanical shaker, after which, while the flour was still in suspension, another 10 ml. aliquot was pipetted off and used to make a second 0.01 dilution. The flour in the 0.1 dilution was then allowed to settle for approximately ten minutes before pipetting off 10 ml. of the supernatant liquid which was used to make a third 0.01 dilution. Ten replicate plates were prepared from each of the three 0.01 dilutions. The medium used in five of the plates was acidified Czapek's agar and in the remaining five plates

Christensen's malt-salt agar. Incubation was at 25 degrees C. for six days.

The raw counts obtained in this study and certain data necessary for an analysis of variance are presented in Appendix A. Section III. The counts were tested for the following:

1. Significant differences between methods,
2. Significant difference between media, and
3. Significant differences between combinations of methods and media. The results are shown in Table 3.

Table 3. Effects of method of treating the initial dilution and kind of medium on the count of fungi in flour.

Source of variation	Degrees freedom	Mean square	F. value	F.at 5%
Samples	9	2157.79		
Methods	2	4239.88	261.24	3.04
Media	1	174.80	10.77	3.89
Samples x methods	18	314.12		
Samples x media	9	8.25		
Methods x media	2	33.97	2.09	3.04
Smpl.x meth.x media	18	25.29		
Error	240	16.23		

Necessary difference for methods = 1.120 at the 5% level.
Necessary difference for media = 0.914 at the 5% level.

Table 3 shows that different methods of treating the initial dilution of a sample of flour yielded signficantly different counts. The mean count obtained from 100 plates when the dilution was shaken for two minutes was 12.95 and when shaken for thirty minutes 14.83 (app.A. sec.III.). On the basis of a necessary difference of 1.12* between these means it is evident that a significantly higher count of fungi is obtained when the initial dilution is shaken thirty minutes than when it is shaken two minutes. When aliquots of the uniformly suspended flours were pipetted immediately after shaking, the mean count obtained from 100 plates was 14.83 and when the supernatant liquid was used the mean count was 2.73. On the basis of the same necessary difference between means it is evident that pipetting should be done immediately after the samples are removed from the shaker.

Further it may be noted that the two media yielded significantly different counts. In the case of Czapek's agar the mean count from 150 plates was 14.11 and in the case of malt-salt agar it was 16.40 (app.A. sec.III.).

This table also shows that combining any medium with methods of treating the initial dilution did not produce an effect other than that attributable to either medium or methods.

* at the 5% level.

THE MICROFLORA IN COMMERCIALLY MILLED FLOUR

An investigation was made on the numbers and types of micro-organisms in fifty samples of commercially milled fresh flour. Duplicate samples of the same five brands of commercial flour were obtained every two weeks, over a period of eight weeks, from a commercial milling company in Winnipeg. The five brands, designated 1,2,3,4 and 5 represented flours of different quality milled by this company. The results of a routine mill analysis for ash, protein and moisture on each brand for each date of sampling were provided by J. A. Hessel, the mill chemist. These may be found in Appendix B, Section I. The samples were taken in sterile, 6 ounce, wide mouth, screw-top jars direct from the flour streams in the mill. They were plated on the following day.

Method of Procedure

Each sample was thoroughly mixed on a large sheet of sterile paper by following a standard procedure. A 10 gram portion was then weighed in a small pyrex beaker (flamed and cooled previous to use) on a balance accurate to 0.01 of a gram and transferred to a 6 ounce screw-top bottle containing 93 ml. of water and a small quantity of fine gravel. This dilution was shaken for thirty minutes on a mechanical shaker. Afterwards the dilution was shaken an additional

twenty-five times by hand and then agitated continuously while a 10 ml. aliquot was pipetted to a 90 ml. water blank. This represented the 0.01 dilution. Samples 3, 4, and 5 represented poorer quality flours, which necessitated further diluting for plate count studies. In these cases the 0.01 dilution was shaken twenty-five times and a 10 ml. aliquot was transferred to a second 90 ml. blank - thus giving the 0.001 dilution.

These dilutions were used for preparing triplicate plates for each of:

1. Bacteria on nutrient agar at 25 degrees C. Many investigators (1,7,8,11) have used 37 degrees C. for this determination. Since it might be expected that the bacterial flora of flour under normal conditions would seldom be exposed to this high temperature, it appeared more reasonable to use the lower temperature of incubation.
2. Bacteria on brom-cresol-purple dextrose tryptone agar at 25 degrees C. This medium was used primarily to obtain acid producing mesophilic bacteria.
3. Fungi and yeasts on Czapek's agar at 25 degrees C. and
4. Fungi on malt-salt agar at 25 degrees C. Counts on this medium were obtained in order to provide additional data for purposes of comparison with counts on Czapek's medium.

Immediately after pipetting to the above plates each 0.01 dilution was heated at 90 degrees C. for thirty minutes. After shaking twenty-five times by hand the heated dilution was used

for the following:

5. Plate counts of thermophilic flat sour spores by a procedure developed for studies on sugar (14). One ml. from the heated 0.01 dilution was placed in each of ten plates. Brom-cresol-purple dextrose tryptone agar was added. Incubation was at 55 degrees C. for forty-eight hours. A count of the acid producers developing on the ten plates at this dilution multiplied by ten represented the numbers of thermophilic flat sour spores per gram.

6. Plate counts of thermophilic spores. All the colonies developing on the plates referred to immediately above were counted for this purpose.

7. Counts of anaerobic thermophilic spores not producing hydrogen sulphide by a technique likewise developed for sugar. These were grown in Bacto-liver broth prepared by boiling 50 grams of Bacto-liver in 500 ml. of water for one half an hour and then filtering. The filtered solution was added to a solution of 10 grams of peptone and 1 gram of dipotassium hydrogen phosphate (K_2HPO_4) dissolved in 500 ml. of water. The combined solutions were brought to a total volume of 1 litre and adjusted to a pH of 7 before adding 1 ml. of brom-cresol-purple. The medium was tubed (5 ml. per tube) and sterilized. The tubed medium was heated at 90 degrees C. for thirty minutes immediately before using. Two ml. from the heated 0.01 dilution were added to each of ten tubes containing 5 ml. of the previously heated Bacto-liver broth, then

2 ml. of sterile liquid petrolatum were added to each tube. Incubation was at 55 degrees C. for seventy-two hours. The production of acid in a tube was taken as being indicative of the presence of one spore. A count of the positive tubes from this dilution multiplied by five represented the number of anaerobic thermophilic spores per gram. And

8. Counts of spores of rope bacteria obtained by a variation of the method used by Kent-Jones and Amos (12). One ml. of the heated 0.01 dilution was added to each of ten tubes containing 5 ml. of nutrient broth. Incubation was at 37 degrees C. for seventy-two hours. The tubes were shaken, in order to mix the contents, after twenty-four hours incubation. The formation of a pellicle in a tube was taken as being indicative of the presence of one spore of rope bacteria. A count of the tubes showing pellicle formation from this dilution multiplied by ten represented the number of spores per gram.

Results

The average number of each type of micro-organism per gram for the five brands of flour is shown in Table 4. The counts (obtained directly from the dilutions used for this purpose) for each type of micro-organism from each sample may be found in Appendix B. Section II.

The same data expressed as logarithms of numbers are presented graphically in Fig. 1 and 2.

Table 4. Average numbers of micro-organisms per gram in commercially milled flours.*

Type of micro-organism#	Brands 1	2	3	4	5
1	2750	7980	17630	12900	19500
2	2010	6600	13990	8700	16600
3	39	555	526	1760	720
4	47	60	822	133	196
5	22	24	176	63	72
6	1	0	2	1	2
7	49	36	84	66	85
8	1270	1140	3510	2630	3960
9	1150	1040	2260	2180	2850
10	54	268	12	47	81

* Each figure based on counts from 10 samples.

\# 1. Mesophilic bacteria on nutrient agar.
2. Mesophilic bacteria on B.C.P. dextrose typtone agar.
3. Acid producing mesophiles on B.C.P. dextrose tryptone agar.
4. Spores of thermophilic bacteria.
5. Spores of thermophilic flat sours.
6. Spores of anaerobic thermophiles not producing H_2S.
7. Spores of rope bacteria.
8. Fungi on malt-salt agar.
9. Fungi on Czapek's agar.
10. Yeasts.

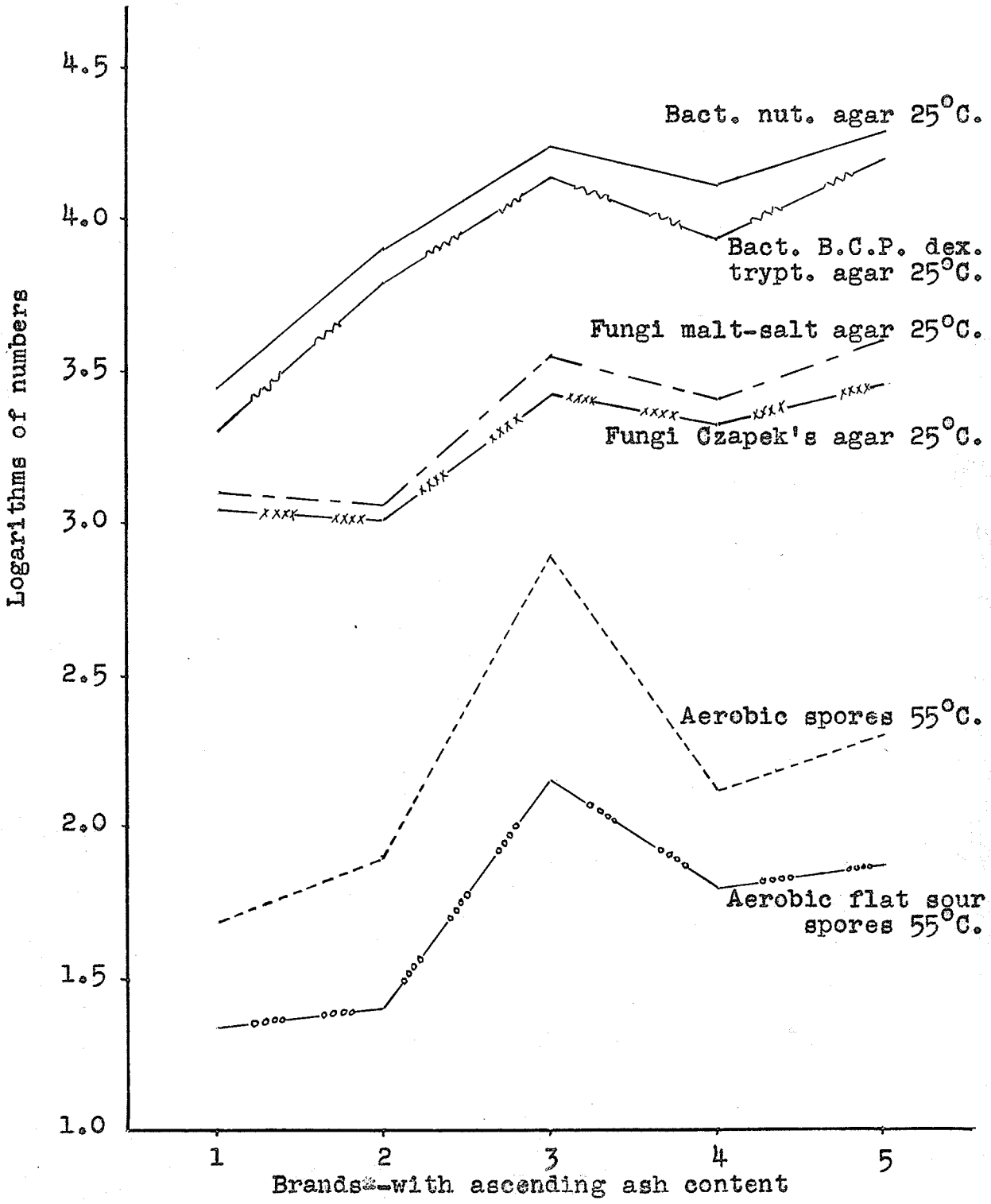

Fig. 1 Relation between average numbers of six types of micro-organisms of flour studied and brands.

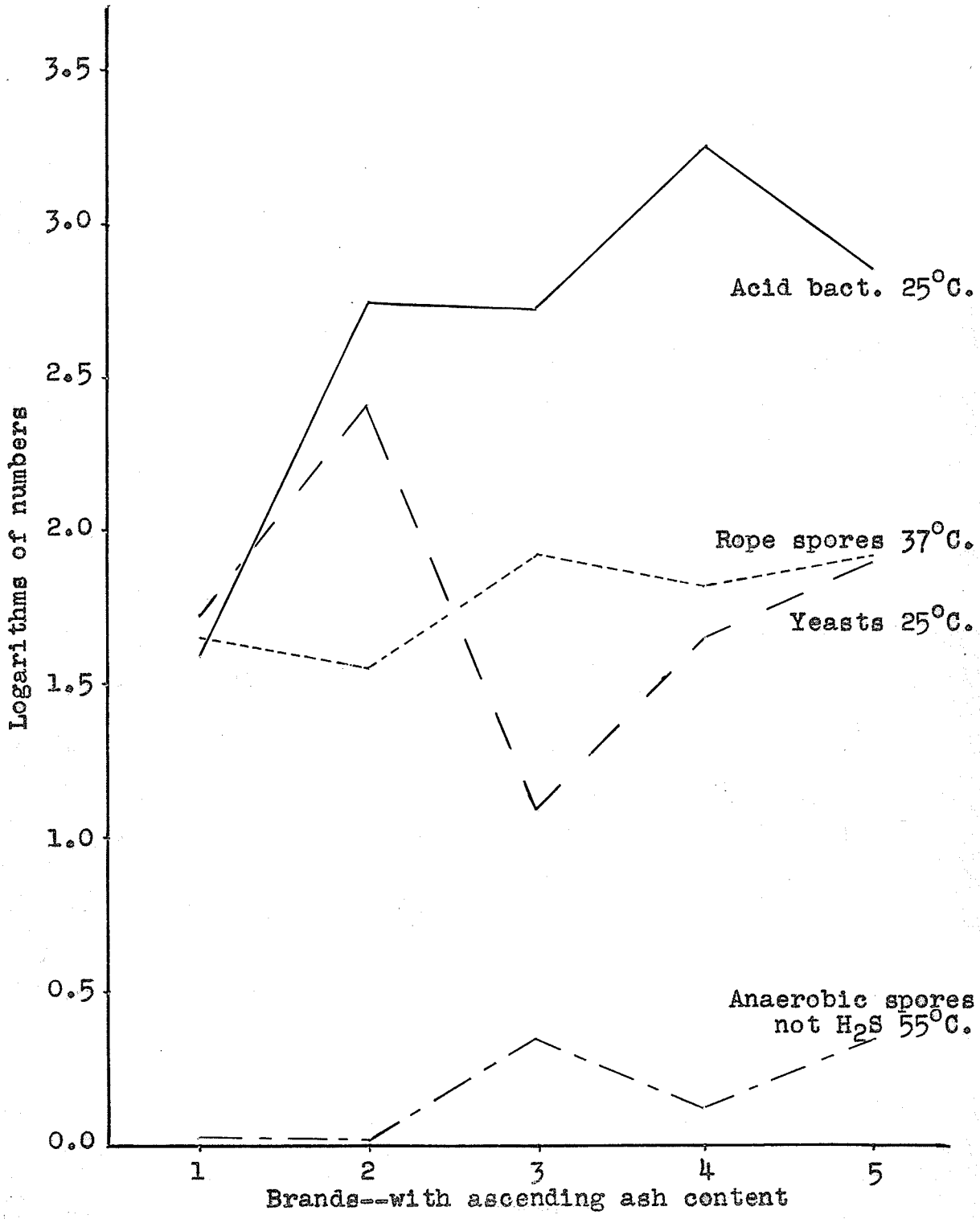

Fig. 2 Relation between average numbers of four types of micro-organisms of flour studied and brands.

The raw data from the dilutions counted for (a) mesophilic bacteria on nutrient agar (b) fungi on malt-salt agar and (c) thermophilic aerobic spores were submitted to an analysis of variance test for significant differences between brands. These groups were chosen as representative of the three levels of counts that show a similar trend (see Fig. 1). The results are shown in Tables 5, 6 and 7.

Table 5. Significance of variation in counts of mesophilic bacteria on nutrient agar.

Source of variation	Degrees freedom	Mean square	F. value	F.at 5%
Two week intervals	4	643.40	22.03	2.76
Brands	4	454.85	15.58	2.76
Intervals x brands	16	168.44	5.77	
Error	25	29.20		

Necessary difference for intervals = 4.99 at the 5% level.
Necessary difference for brands = 4.99 at the 5% level.

Table 6. Significance of variation in counts of fungi on malt-salt agar.

Source of variation	Degrees freedom	Mean square	F. value	F.at 5%
Two week intervals	4	145.62	16.73	2.76
Brands	4	1632.77	187.67	2.76
Intervals x brands	16	197.27	22.67	
Error	25	8.70		

Necessary difference for intervals = 1.32 at the 5% level.
Necessary difference for brands = 1.32 at the 5% level.

Table 7. Significance of variation in counts of thermophilic aerobic spores.

Source of variation	Degrees freedom	Mean square	F. value	F.at 5%
*Two week intervals	3	1782.42	16.28	3.10
Brands	4	9855.47	90.02	2.87
Intervals x brands	12	2025.01	18.50	
Error	20	109.48		

Necessary difference for intervals = 9.78 at the 5% level.
Necessary difference for brands = 10.93 at the 5% level.

*At the fourth interval organisms did not develop on any plate from any brand at this temperature in this medium. This could not be attributed to medium since colonies developed on it at 25 degrees C. Further, the temperature of heating could not have destroyed all spores since spores of rope bacteria developed in transfers from the same dilution bottles. This analysis accordingly was confined to the data obtained on four dates of sampling only.

In order to obtain additional evidence of the difference between the two media used for counts of fungi (see table 3), the following analysis was carried out. In this case the raw counts of fungi obtained on each sample from each brand on each medium were used. The analysis is shown in Table 8.

Table 8. Effects of kind of medium on the count of fungi in flour.

Source of variation	Degrees freedom	Mean square	F. value	F.at 5%
Media	1	750.76	10.50	3.94
Brands	4	2273.03		
Error	94	71.49		

Discussion

The brands 1 to 5 represent progressively poorer qualities of flour. The more highly refined a flour is the less ash it contains (see Appendix B. Section I). On this basis the brands 1 to 5, excepting brand 3, follow an expected trend in relation to all of the groups of micro-organisms shown in Fig. 1: i.e. the micro-organic content increases as the quality of the flour decreases. Brand 3 contains a larger number of micro-organisms than would be expected. Probably this could be explained on the basis of some difference in the processing of

this brand.

Mesophilic bacteria were present in exceedingly large numbers in all brands when compared with other types of micro-organism. A higher count of these organisms was obtained on nutrient agar than on B.C.P. dextrose tryptone agar but both media yielded counts that followed the same trend (Fig.1).

Acid producing mesophilic bacteria did not follow the same general trend from brand to brand as the above types but, as shown in Fig. 2, the poorer quality flours harboured a larger population than did the better quality flours. These organisms under favorable conditions of moisture and temperature could be responsible for the development of sourness and rancidity that has been noted from time to time in flour (7,8).

On the basis of the foregoing it would seem desireable to plate flour samples on B.C.P. dextrose tryptone agar at 25 degrees C. A count of mesophilic bacteria is obtained which, although not as high as that obtained on nutrient agar, indicates the relative populations in the various samples. In addition, the plates can be used to obtain useful information on acid producers.

Fungi constituted the second largest group of micro-organism in all brands. Malt-salt agar in this as well as the preliminary study yielded significantly higher counts than Czapek's agar (Table 9), the mean count on malt-salt agar being 25.04 and on Czapek's agar 19.56. Each medium yielded counts on all brands that followed the same trend. Malt-salt agar is

more easily prepared than Czapek's agar but is more expensive. The different types of fungi are harder to recognize on plates of malt-salt agar.

Most of the species of fungi encountered on this study have been found on wheat (10) or in soil in Manitoba (4,5). The following species represent types not reported in these references. P. canescens Sopp, P. casei Staub, P. citreo-sulfuratum Biourge, P. matris-meae Zaleski, P. notatum Westling, P. pfefferianum (Wehmer) Westling, P. roseo-maculatum? Biourge, P. urticae Banier. One species Penicillium urticae Banier occurred on practically all plates. Evidently, this species represents a common contaminant in this mill.

Spores of thermophilic aerobes and of flat sours were the third and fourth most numerous types showing the same trend as the mesophilic bacteria and fungi. On many plates they produced spreader colonies. Under certain conditions these organisms could cause trouble in baked products.

Spores of thermophilic anaerobes were present in such small numbers that their effect probably would be negligible except under specific conditions unlikely to occur in flour.

The yeast count was small and fluctuated from brand to brand without showing the same trend as other types (Fig.2). A high count of unknown strains of yeast conceivably might affect fermentation during the leavening process.

The count of rope spores also fluctuated from brand to brand. The poorer quality flours contained on the whole more

spores than the better quality flours. An experiment was carried out using 78 isolates to determine if all the pellicle forming organisms belonged to one species. The isolates were tested for acid production from xylose, arabinose, dextrin and mannitol. The results showed that 36 of the isolates produced acid from arabinose, mannitol and dextrin and no acid from xylose and on the same basis thirteen of the cultures arranged themselves into various other groups. None of the above groups agreed with any known species of the genus Bacillus. Fourteen of the isolates agreed with the description of B. pumilis, thirteen with B. subtilis and two with B. panis on the basis of reaction in sugars (3). Therefore, it appears evident that a reliable estimate of the number of rope bacteria spores was not obtained with the technique used. Further investigation is needed to develop a reliable method for obtaining a count of spores in flour.

Significant differences between certain brands were evident for mesophilic bacteria, for fungi and for thermophilic aerobic spores, as the trends in Fig. 1 indicate.

The mean counts of mesophilic bacteria in all brands at the two-week intervals were 21.0, 18.8, 13.0, 2.6 and 5.6 (app.B,Sec.II). On the basis of a necessary difference of 4.99* it is evident that the count on each date of sampling differed significantly from the counts on at least three other dates of sampling. This was true also for fungi and thermophilic aerobic spores. However no uniform significant

* at the 5% level.

variation from date to date of sampling occurred for any two of the three types of organisms. Therefore, it would not be advisable to place reliance on the count obtained from one day's sampling as being indicative of the average microbial population of any brand. These variations are probably due to differences that occur from time to time in the microfloral content of the mill mix. However a count of specific type of organism that was consistently higher over a series of samples than its predetermined average in the brand would probably indicate an abnormal condition.

SUMMARY

1. Estimates of the numbers of bacteria, fungi and yeasts were made on milled products obtained at various stages in the milling process of two samples of wheat. The products showed a definite but irregular decrease in numbers of organisms as the amount of seed coat in the product decreased.

2. Counts of fungi were made on replicate samples, replicate portions of samples and replicate dilutions. A significant variation was found between replicate samples only.

3. Significantly higher counts of fungi were obtained when the initial dilution was shaken thirty minutes rather than two minutes. Further, higher counts were obtained when a transfer from the initial dilution was made immediately after shaking - i.e. before settling.

4. Malt-salt agar was found to yield a significantly higher count of fungi from flour than Czapek's agar.

5. Estimates of numbers of certain types of bacteria, of fungi and of yeast were made on five brands of flour obtained from a milling company at bi-weekly intervals. In general, counts of most types were lowest in brands with low ash

content. Mesophilic bacteria constituted by far the greatest proportion of the microbial population. Other types occurred in the following order: fungi, acid producing mesophiles, spores of thermophilic bacteria, spores of thermophilic flat sours, rope spores, yeasts and spores of anaerobic thermophiles not producing H_2S.

6. P. urticae was found on practically all samples.

7. Spores of rope bacteria isolated by the recognized method were found to represent several species. Only relatively few isolates belonged to either of the species reported to be the cause of ropiness in bread.

8. Numbers of organisms of the various types were found to vary significantly with dates of sampling.

REFERENCES

(1) Barton-Wright, E. C. Studies on the storage of wheat flour. Changes in flora and fats and influence of these changes on gluten character. Cereal Chem. 15: 521-541. 1938.

(2) Barton-Wright, E. C. and Tomkins, R. G. The moisture content and growth of mould in flour, bran and middlings. Cereal Chem. 17: 332-342. 1940.

(3) Bergey, D. H. Bergey's manual of determinative bacteriology. 5th ed: 643, Baltimore, The Williams and Wilkins Co. 1939.

(4) Bisby, G. R., James, N. and Timonin, M. Fungi isolated from Manitoba soil by the plate method. Can. J. Research, 8: 253-275. 1933.

(5) Bisby, G. R., Timonin, M. I. and James, N. Fungi isolated from soil profiles in Manitoba. Can. J. Research, C,13: 47-65. 1935.

(6) Christensen, C. M. The quantitative determination of molds in flour. Cereal Chem. 23: 322-329. 1946.

(7) Gustafson, C. B. and Parfitt, E. H. Effect of numbers of bacteria on the development of rancidity in soft wheat flour. Cereal Chem. 10: 233-238. 1933.

(8) Holtman, D. F. The microbial content of soft wheat flour. J. Bact. 30: 359-361. 1935.

(9) James, N. and Sutherland, M. L. The accuracy of the plating method for estimating the numbers of soil bacteria, actinomycetes and fungi in the dilution plated. Can. J. Research, C.17: 72-86. 1939.

(10) James, N., Wilson, W. and Stark, E. The microflora of stored wheat. Can. J. Research, C.24: 224-233. 1946.

(11) Kent-Jones, D. W. and Amos, A. J. Preliminary studies in the bacteriology of wheat and flour. Analyst, 55: 246-268. 1930.

(12) Kent-Jones, D. W. and Amos, A. J. Studies in the bacteriology of flour. Analyst 56: 573-585. 1931.

(13) Amos, A. J. The microbiology of cereals. Ch. XIV. In Kent-Jones, D. W. Modern cereal chemistry. 3rd ed. Liverpool, The Northern Publishing Co. Ltd. 1939.

(14) -------Official and tentative methods of association of official agricultural chemists. 6th ed: 753-755. Washington, D. C. Association of official agricultural chemists. 1945.

(15) Tanner, F. W. The microbiology of foods. 2nd ed: 697-715. Champaign, Ill. Garrard Press. 1944.

(16) -------The Canada year book. Ottawa, Edmond Cloutier, Kings Printer and controller of stationery XX. 1947.

APPENDIX A. PRELIMINARY STUDIES

Section I Experimentally milled samples

Sample 1

Bacteria Repls.		Milled products*								
		1	2	3	4	5	6	7	8	9
1	1	20	81	91	53	45	27	128	154	146
	2	33	85	75	49	63	23	174	147	129
2	1	48	98	90	43	73	30	46	159	101
	2	91	91	93	55	87	25	54	142	134
3	1	91	57	27	37	50	25	42	194	121
	2	66	43	17	49	46	27	49	177	68
4	1	31	28	34	24	34	24	44	87	93
	2	39	36	28	29	30	30	45	71	91
5	1	25	20	45	26	29	19	22	31	76
	2	36	19	31	25	21	26	25	38	75
6	1	16	78	32	14	31	20	30	20	81
	2	17	63	33	16	35	25	43	31	81
7	1	18	29	33	25	24	19	25	440	92
	2	18	24	33	29	33	21	28	300	92
8	1	197	57	41	30	18	16	26	82	82
	2	194	54	38	29	32	23	26	79	86
9	1	33	37	28	23	31	18	44	92	238
	2	38	35	25	18	26	18	43	98	217
10	1	61	36	28	23	52	13	21	124	194
	2	46	33	23	19	55	17	23	125	181
Avge. x 10^4		55.9	50.2	42.2	30.8	40.7	22.3	4.7	1.3	1.2
Diln.		10^4	10^4	10^4	10^4	10^4	10^4	10^3	10^2	10^2

Sample 1

Fungi Repls.		Milled products* 1	2	3	4	5	6	7	8	9
1	1	22	6	22	18	8	5	2	0	1
	2	28	2	7	28	5	4	0	0	1
2	1	19	15	4	6	3	1	2	2	0
	2	20	23	17	8	4	5	0	0	0
3	1	25	5	12	21	5	8	0	0	1
	2	28	5	9	22	7	5	0	0	3
4	1	4	4	21	9	9	5	3	0	2
	2	10	12	21	6	8	2	0	2	2
5	1	9	5	33	8	8	6	2	6	1
	2	14	3	35	11	5	12	4	1	2
6	1	18	9	21	13	16	8	6	4	0
	2	11	6	23	11	24	2	4	4	3
7	1	13	6	9	8	15	2	3	2	1
	2	15	3	7	10	9	3	2	2	1
8	1	7	2	10	2	12	2	7	2	0
	2	10	4	5	8	6	5	3	5	1
9	1	6	0	6	3	16	5	7	0	5
	2	8	4	2	9	8	3	5	1	4
10	1	8	6	16	2	15	3	6	0	0
	2	18	3	9	4	17	0	6	3	1
Diln.		10^1	10^1	10^1	10^1	10^1	10^1	10^1	10^1	10^1
Avge. x 10^1		14.6	6.1	14.4	10.4	10.1	4.3	3.1	1.7	1.4

Sample 1

Yeasts	Repls.	Milled products* 1	2	3	4	5	6	7	8	9
1	1	143	--	38	47	87	162	56	99	71
	2	191	113	32	47	92	107	44	121	153
2	1	132	107	28	81	49	127	27	12	104
	2	154	172	41	91	56	134	30	8	151
3	1	113	83	60	43	23	420	131	26	44
	2	143	62	65	40	29	370	107	30	82
4	1	144	181	27	24	15	510	80	17	31
	2	108	165	20	21	22	480	88	21	27
5	1	38	52	76	30	12	150	74	16	25
	2	34	39	58	25	22	140	89	16	34
6	1	72	35	30	17	42	240	42	26	45
	2	33	50	21	15	65	150	60	21	21
7	1	15	10	10	4	4	16	21	26	28
	2	29	11	9	3	2	33	26	25	28
8	1	73	54	4	3	3	12	48	9	21
	2	57	81	7	2	2	15	54	5	13
9	1	125	22	13	2	12	--	20	12	25
	2	124	20	17	2	14	--	21	10	28
10	1	114	22	13	2	26	--	19	9	16
	2	93	24	16	2	27	--	19	11	12
Diln.		10^2	10^2	10^2	10^2	10^2	10^1	10^1	10^1	10^1
Avge. x 10^2		91.7	65.1	29.2	25.0	30.2	17.0	5.3	2.6	4.9

Sample 2

Bacteria Repls.		Milled products* 1	2	3	4	5	6	7	8	9
1	1	68	72	68	51	38	11	27	49	39
	2	75	77	51	48	32	14	34	40	38
2	1	58	35	50	61	33	9	8	35	28
	2	68	28	46	71	29	12	6	27	29
3	1	31	14	73	34	19	11	22	35	66
	2	24	20	54	32	21	12	18	29	70
4	1	19	29	71	45	32	6	21	20	75
	2	10	31	56	65	25	8	26	16	60
5	1	52	11	20	33	18	5	20	57	113
	2	49	9	20	30	16	6	19	58	89
6	1	32	23	39	28	25	6	31	39	34
	2	30	18	36	27	18	6	32	34	46
7	1	133	20	19	13	14	4	37	28	27
	2	124	19	21	16	15	4	27	26	25
8	1	188	12	27	12	16	4	22	28	54
	2	182	17	28	20	11	4	23	24	46
9	1	107	16	14	13	6	2	14	10	20
	2	102	13	14	13	6	2	12	12	18
10	1	22	14	17	18	11	4	9	11	22
	2	24	16	14	13	13	3	8	13	27
Diln.		10^4	10^4	10^4	10^4	10^4	10^4	10^3	10^2	10^2
Avge n x 10^4		69.9	24.7	36.9	32.2	19.9	6.7	2.1	0.3	0.5

Sample 2

Fungi Repls.		Milled products* 1	2	3	4	5	6	7	8	9
1	1	29	27	31	17	18	13	7	1	3
	2	32	16	32	18	9	16	5	0	3
2	1	34	12	25	17	6	20	3	0	1
	2	33	10	30	16	10	14	1	1	0
3	1	35	15	29	14	30	7	4	1	3
	2	26	19	30	17	26	7	3	2	2
4	1	37	12	50	13	71	14	5	2	3
	2	34	8	63	10	25	15	14	2	3
5	1	31	7	26	4	34	2	1	2	0
	2	23	10	21	8	34	4	0	0	1
6	1	28	9	49	1130	8	4	4	1	6
	2	34	8	70	930	5	2	1	4	6
7	1	38	18	79	8	10	18	4	4	6
	2	36	15	107	9	9	14	6	4	4
8	1	39	12	53	58	22	12	6	3	4
	2	26	6	77	61	42	9	7	2	10
9	1	23	5	15	4	33	7	8	2	2
	2	25	13	58	6	70	11	7	0	2
10	1	73	78	750	16	23	5	1	1	4
	2	74	75	910	11	42	8	1	1	6
Diln.		10^1	10^1	10^1	10^1	10^1	10^1	10^1	10^1	10^1
Avge x 10^1		35.5	18.7	125.5	118.4	26.4	10.1	4.4	1.7	3.5

Sample 2

Yeasts Repls.		Milled products* 1	2	3	4	5	6	7	8	9
1	1	325	71	38	124	164	139	20	48	50
	2	317	61	--	--	192	158	20	15	--
2	1	183	110	103	69	370	115	36	83	20
	2	161	115	104	58	375	119	34	42	--
3	1	143	38	17	160	13	38	54	8	3
	2	128	23	17	76	10	41	40	7	5
4	1	111	26	7	59	29	28	35	7	10
	2	95	--	6	--	--	33	30	6	9
5	1	33	18	6	13	21	6	31	7	11
	2	17	21	5	6	33	7	24	4	10
6	1	113	13	3	--	12	4	20	4	5
	2	68	10	4	--	9	2	20	3	7
7	1	39	4	9	270	40	11	14	4	7
	2	40	5	6	290	50	15	16	3	7
8	1	116	5	4	220	22	12	24	4	3
	2	170	6	5	270	28	9	22	3	4
9	1	50	3	4	7	5	3	7	2	3
	2	37	-	2	6	8	0	10	2	9
10	1	26	3	-	8	1	2	5	2	4
	2	29	2	-	3	3	3	8	2	5
Diln.		10^2	10^2	10^2	10^1	10^1	10^1	10^1	10^1	10^1
Avge x 10^2		112.6	29.7	20.0	10.2	7.3	3.7	2.4	1.3	1.0

* 1- Wheat
2- Bran
3- Bran chips
4- Coarse shorts
5- Fine shorts
6- Feed flour
7- 1st clear flour
8- 1st patent flour
9- 2nd patent flour

Section II Sampling procedure

First day

Replicate samples	Replicate dilutions	Replicate plates 1	2	3	4	5
1	1	18	18	16	15	14
	2	21	20	20	20	23
	3	18	22	15	13	17
	4	16	23	15	17	20
2	1	14	16	19	14	19
	2	17	18	24	18	14
	3	22	16	21	20	19
	4	15	9	11	15	20
3	1	15	17	22	11	24
	2	20	15	19	15	13
	3	16	16	19	22	18
	4	23	13	12	18	18
4	1	18	18	23	13	19
	2	14	19	20	17	19
	3	19	17	16	18	17
	4	13	25	21	15	17

Second day

Replicate samples	Replicate dilutions	Replicate plates 1	2	3	4	5
1	1	20	15	12	19	15
	2	19	19	15	17	16
	3	21	11	13	15	20
	4	17	17	13	17	16
2	1	18	12	13	15	13
	2	10	14	15	19	17
	3	16	23	14	20	10
	4	22	13	16	17	10
3	1	12	17	14	15	17
	2	14	20	20	18	10
	3	15	16	16	21	15
	4	13	19	22	14	12
4	1	14	19	15	21	13
	2	8	16	13	18	21
	3	17	14	17	19	19
	4	14	15	15	17	23

Third day

Replicate samples	Replicate dilutions	Replicate plates 1	2	3	4	5
1	1	10	13	14	9	14
	2	8	18	13	16	15
	3	17	12	18	14	16
	4	13	19	13	11	20
2	1	10	15	12	16	14
	2	16	21	23	11	13
	3	19	20	11	14	11
	4	11	11	17	16	11
3	1	10	16	14	20	20
	2	12	6	11	13	25
	3	12	13	14	15	14
	4	12	19	8	15	15
4	1	10	12	15	14	14
	2	16	16	16	15	13
	3	17	18	11	20	15
	4	14	17	11	14	14

Section III Methods and media

Samples	Replicate plates	Method 1 Cz.	Method 1 M-s.	Method 2 Cz.	Method 2 M-s.	Method 3 Cz.	Method 3 M-s.
1	1	4	6	5	10	1	2
	2	6	7	6	11	1	0
	3	3	3	6	9	1	0
	4	0	3	6	4	0	1
	5	4	6	5	1	0	3
2	1	21	25	21	27	1	4
	2	22	29	20	29	3	3
	3	23	26	26	25	4	3
	4	23	30	23	28	8	4
	5	19	25	21	26	0	2
3	1	6	11	10	6	2	3
	2	5	12	10	19	4	3
	3	4	10	10	30	0	2
	4	12	7	6	9	1	4
	5	14	4	16	12	0	2
4	1	0	3	3	7	0	3
	2	6	2	3	7	1	3
	3	3	1	2	2	0	1
	4	2	4	4	1	0	2
	5	3	5	1	1	1	3
5	1	11	9	18	14	2	3
	2	11	12	11	28	1	6
	3	17	10	12	16	1	1
	4	10	12	15	14	9	2
	5	6	20	8	13	3	3

Samples	Replicate plates	Method 1* Cz.	Method 1* M-s.	Method 2* Cz.	Method 2* M-s.	Method 3* Cz.	Method 3* M-s.
6	1	43	33	45	44	9	6
	2	42	37	33	47	11	13
	3	46	49	43	49	6	6
	4	43	34	40	52	6	10
	5	30	39	56	54	7	7
7	1	6	12	12	7	0	5
	2	9	27	13	11	1	0
	3	7	15	11	17	3	1
	4	6	11	23	17	2	3
	5	6	14	22	11	3	2
8	1	5	10	17	3	2	6
	2	7	16	15	15	7	1
	3	9	4	6	7	5	1
	4	9	4	6	7	5	1
	5	9	10	7	11	1	0
9	1	4	7	7	3	0	0
	2	4	11	11	11	1	0
	3	9	11	9	11	2	1
	4	8	12	3	8	0	3
	5	5	3	4	9	1	0
10	1	11	11	5	8	0	2
	2	9	9	7	6	2	0
	3	5	11	2	19	3	2
	4	9	17	6	12	1	1
	5	25	11	9	13	2	6

* Method 1 Initial dilution shaken two minutes
Method 2 Initial dilution shaken thirty minutes and flour pipetted immediately
Method 3 Initial dilution shaken thirty minutes and flour pipetted after settling

APPENDIX B. THE MICROFLORA IN COMMERCIALLY MILLED FLOUR

Section I Results of routine analysis on flour samples

Date of sampling	Brand No.	Ash	Moisture	Protein
1	1	.33	13.5	11.6
	2	.35	14.1	12.0
	3	.47	13.6	13.9
	4	.52	13.6	13.4
	5	.58	14.2	15.7
2	1	.32	13.5	11.2
	2	.35	13.6	13.0
	3	.43	13.7	14.0
	4	.58	13.9	14.6
	5	.59	13.5	16.4
3	1	.32	13.9	11.2
	2	.35	14.3	12.2
	3	.44	13.8	13.4
	4	.46	14.1	12.8
	5	.58	13.6	15.0
4	1	.33	13.8	11.1
	2	.38	13.8	12.3
	3	.44	13.8	13.1
	4	.58	13.9	14.0
	5	.62	14.0	14.6
5	1	.32	13.5	11.2
	2	.34	14.0	12.0
	3	.44	13.7	13.5
	4	.52	14.1	12.9
	5	.64	14.0	15.0

Section II Raw counts for types of micro-organisms from each sample of commercial flour

	Organism*	Two week intervals 1 Repl. 1	1 Repl. 2	2 Repl. 1	2 Repl. 2	3 Repl. 1	3 Repl. 2	4 Repl. 1	4 Repl. 2	5 Repl. 1	5 Repl. 2
Brand 1	1	3	4	3	7	7	1	1	2	1	2
	2	2	4	3	3	5	1	1	1	1	1
	3	0	0	0	0	0	0	0	1	0	0
	4	7	12	6	3	7	3	0	0	5	4
	5	2	1	1	5	5	1	0	0	3	3
	6	0	0	0	0	0	0	0	0	0	0
	7	7	6	2	0	2	4	9	5	4	10
	8	20	14	7	5	11	8	13	13	17	19
	9	15	13	4	6	11	8	13	11	17	17
	10	0	2	1	2	1	1	1	2	0	0
Brand 2	1	17	7	17	11	10	9	1	3	2	3
	2	13	5	16	10	8	8	1	2	2	3
	3	3	2	0	0	0	0	1	2	2	3
	4	9	15	6	3	10	12	0	0	2	3
	5	3	3	4	1	2	5	0	0	2	3
	6	0	0	0	2	0	0	0	0	0	0
	7	1	2	7	2	2	4	4	4	3	7
	8	8	7	8	10	13	11	9	17	14	17
	9	7	4	7	11	12	11	8	7	20	17
	10	11	12	1	1	1	1	1	1	0	1
Brand 3	1	53	45	6	13	34	10	2	2	4	7
	2	42	41	5	6	26	8	6	1	7	4
	3	0	0	1	2	0	0	0	0	1	2
	4	67	56	152	103	14	8	0	0	180	142
	5	17	12	21	44	9	3	0	0	33	37
	6	0	0	1	2	0	0	0	0	0	1
	7	9	10	10	10	4	3	8	10	10	10
	8	54	52	21	24	29	32	33	29	36	41
	9	39	38	17	32	24	26	20	20	28	32
	10	0	0	0	0	1	1	0	0	0	0

Organism*	Two week intervals 1 Repl. 1	1 Repl. 2	2 Repl. 1	2 Repl. 2	3 Repl. 1	3 Repl. 2	4 Repl. 1	4 Repl. 2	5 Repl. 1	5 Repl. 2
Brand 4										
1	25	15	33	23	12	6	3	3	3	6
2	22	14	11	14	11	7	2	1	2	3
3	2	1	4	1	3	4	1	1	1	1
4	17	22	31	18	11	12	0	0	11	11
5	7	8	18	9	6	5	0	0	3	7
6	1	0	0	0	1	1	0	0	0	0
7	7	6	9	7	3	4	5	9	8	8
8	15	16	40	30	23	30	28	25	29	28
9	9	8	29	20	23	19	20	22	24	24
10	1	0	1	0	1	2	0	0	1	1
Brand 5										
1	28	13	36	39	17	24	5	5	11	17
2	23	14	39	33	10	16	3	5	8	15
3	0	2	0	1	1	2	1	1	1	0
4	53	51	12	12	11	10	0	0	27	20
5	18	6	8	10	4	3	0	0	16	7
6	1	0	0	1	1	2	0	0	0	0
7	10	10	9	9	10	7	6	8	10	6
8	20	17	30	38	58	58	35	35	52	52
9	14	15	19	19	35	39	32	29	42	41
10	1	3	1	0	2	1	0	0	1	0

* 1. Mesophilic bacteria on nutrient agar at 25°C. Each figure based on counts from three plates at the 0.001 dilution.
2. Mesophilic bacteria on B.C.P. dextrose tryptone agar at 25°C. Counted as number 1.
3. Acid producing mesophiles counted on plates of number 2.
4. Spores of thermophilic bacteria.
5. Spores of thermophilic flat sours on plates of number 4.
6. Spores of anaerobic thermophiles not producing H_2S.
7. Spores of rope bacteria.
8. Fungi on malt-salt agar at 25°C. Each figure based on counts from three plates at the 0.01 dilution.
9. Fungi on Czapek's agar at 25°C. Counted as number 8.
10. Yeasts. Counted on plates of number 9.