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The effect of lactobacilli and micrococci cultures on the ripening of dry sausage.

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The use of microbes both as pure cultures and as mixed cultures in the preparation of various food articles as agents intended to influence the quality and flavor of the product and to speed up and stabilize the production process is a practice nearly as old as that of food preparation on the whole. The application of bacterial cultures has been developed very far in the dairy industry. Considerably lesser is the spread which additions of bacterial cultures have gained in the meat-processing industry. The manufacture of fermented meat products, such as dry sausages, is still often dependent on an initial bacterial flora determined by chance.

A. EARLIER INVESTIGATIONS

Several attempts to adopt the use of bacterial cultures also in the meat-processing industry have been made. The first were made in the USA. (DRAKE 1928, JENSEN and PADDOCK 1940, KURK 1921).

Using lactobacilli in pure and mixed cultures, JENSEN and PADDOCK observed that they eliminated faulty characteristics caused by non-desirable bacteria. Moreover, the processing time could be shortened. A high temperature, 35-45°C, was used during the smoking. In 1955, both NIVEN et al. (1955) and NIINIVAARA (1955) presented new conceptions of the use of bacterial pure cultures in the manufacture of fermented meat products. As a suitable bacterial addition to the American dry sausage type, the "summer sausage", NIVEN (1955) recommended a *Pediococcus cerevisiae* strain. NIINIVAARA (1955), again, recommended micrococci as appropriate bacterial additive to dry sausage of European type. Continued investigations into the properties of *Micrococcus* strains suitable to be used in this manner have been carried out by POHJA (1960) and NIINIVAARA et al. (1964). Since around 1957, both kinds of bacterial cultures have been available as lyophilized commercial products for use in manufacturing fermented sausages, namely, *Pediococcus* under the name "ACCEL", produced by MERCK & Co.

RAHWAY, N.J., USA, and M i c r o c o c c u s under the name "BAKTO-FERMENTE", produced by RUDOLF MÜLLER Co., Hamburg, Western Germany. In the USA nowadays 50 per cent of all dry sausages are produced with the aid of various bacterial additions (LEISTNER 1963). The use of a M i c r o c o c c u s culture in the preparation of dry sausage has also gained ground in Europe.

The possibilities for using the said bacterial cultures have been studied and criticized. Kuchling (1963) experimented with M i c r o - c o c c u s strains obtained from NIINIVAARA and POHJA and with strains isolated by himself. He found that the M i c r o c o c c u s addition accelerated the formation of color, but this difference was only noted during the first 24 hours in comparison to sausages processed without bacterial addition. According to him there was no effect on the speed of the ripening process or on the lowering of pH. KUCHLING (1964) noted that the processing time of the European-type dry sausage can be shortened by the addition of a P e d i o - c o c c u s culture. With the aid of bacterial additions, rapid formation of lactic acid and lowering of the pH value can be ascertained from the very beginning. The bacterial addition used by him, on the average 10^{14} bacteria per gram of sausage mixture, is very high and therefore impracticable in actual use owing to high expense.

KUCHLING (1963) observed that the experiments with M i c r o - c o c c u s and P e d i o c o c c u s additions were not fully in support of the results of those who developed the practices. When lactobacilli and pediococci are used, there is a risk of discoloration due to the rapid formation of acid. On the other hand when nitrate-reducing bacteria are used, disturbance of the formation of acid is sometimes claimed to result in faulty products. The reason why continued experiments have partly failed may frequently be that the starter culture strains in question have lost their useful properties. NIVEN (1961) demonstrated, for instance, that the salt tolerance of lyophilized bacteria diminished.

TEN CATE (1960) observes on the strength of his investigations that the production of dry sausage is entirely possible without the addition of bacteria because these are already present in the initial bacterial flora of the sausage mixture. With their aid sufficient formation of lactic acid takes place.

Fairly numerous studies concerning the significance of lactobacilli with respect to the characteristics of dry sausage have been presented. LERCHE (1955) and CORETTI (1956) observe, for instance, that lactobacilli are noted in a very small number in dry sausage mixture. Their number increases during the smoking and ripening period, and they constitute the overwhelmingly dominant part of the bacterial flora in the completed product. Several authorities (e.g. LANG 1960, LERCHE 1955, and SKOVGAARD 1963) observe that lactobacilli must play a considerable role in the ripening process of dry sausage. Opinions have also been presented concerning their significance as actual producers of flavor. On the other hand, several authorities (e.g. CORETTI 1958, LANG 1960, NIVEN et al. 1949, SKOVGAARD 1963) have found that lactobacilli may cause faulty properties in dry sausages, such as color and flavor defects.

LANG (1960) isolated from dry sausage with faulty coloration a L a c t o b a c i l l u s strain and used it when inoculating slices of sausages of Frankfurter type according to the instructions given by ZELLER (1956, 1957). He did not notice any color defect in these within 48 hours.

B. OWN INVESTIGATIONS

I. Materials

The purpose of the study was to clarify the effect of various lactobacilli and of a commercial M i c r o c o c c u s preparation on the ripening process and quality of dry sausage, paying attention also to the faulty characteristics caused by the bacterial additions.

In the present study the dry sausage was prepared in the following manner.

Ingredients:
35 kg beef
35 kg pork
30 kg pork fat
40 g spice mixture
300 cm^3 red wine
100 g phosphate mixture "Kesto sitonal"

Spice mixture:
821 g salt
75 g glucose
12 g nitrate
87 g white pepper
5 g cardamon

In the above-stated proportions usually 20 kg sausage mixture was made and divided into batches of 2.5 kg each. To every batch except the control the bacterial culture to be examined was added before the grinding was finished. The sausages were predried in an AUTOTHERM-cabinet for three days at 18-20°C and 85-95 per cent relative humidity. The temperature during the smoking and ripening process has been 20-22°C and the humidity, 80-90 per cent.

The *Lactobacillus* strains used had been isolated from faulty as well as normal, finished dry sausages. The strains were added in the form of MRS broth cultures (de MAN et al. 1960). The quantity of lactobacilli added varied between 10.5 and 40 million bacilli per gram of sausage mixture. The strains were indentified according to SHARPE (1961) and to BERGEY's Manual (1957). Most of the strains used inoculum were identified as *L. plantarum*; moreover, there was one *L. Leichmannii* and one *L. casei* strain and a few so far unidentified *Lactobacillus* strains. The micrococcal addition was a commercial, lyophilized micrococcal preparation (BAKTOFERMENTE) used in physiological saline solution in five test series and as meat broth culture in two test series.

The quantity of micrococci added was 5-10 million micrococci per gram of the mixture.

The test series comprised the following groups:

1. Control group, no bacterial addition
2. Group with addition of micrococci at 5-10 million per g
3. Group with addition of lactobacilli at 10-40 million per g
4. Group with addition of micrococci (at 5-10 million per g) and lactobacilli (at 10-40 million per g).

II. Methods

1. pH measurement.

2. Organoleptic evaluation

The evaluation was usually performed with sausages 2, 3, 5, 7, 10, 14 and 21 days old, using the following scoring system:

a) Consistency

0 - Completely inferior
1 - Poor
2 - Soft
3 - Rather soft
4 - Fairly firm, finished product
5 - Good, finished product

b) Color of interior and outer surface

0 - Completely inferior; grey, distinct color defect
1 - Poor, motley
2 - Rather poor; reddish but motley
3 - Fair; red coloration but still some motley character
4 - Good, normal red
5 - Attractive, red

c) Flavor

0 - Completely uneatable
1 - Poor
2 - Rather poor
3 - Fair
4 - Good
5 - Very good

d) The odor was only evaluated by the descriptive method, paying particular attention to odors deviating from normal.

3. Bacterial studies

a) Total bacterial count on blood agar, incubation 2 days at 30°C

b) M i c r o c o c c u s count on blood agar

c) Total bacterial count on MRS agar (de MAN et al. 1960), incubation 2 days at 30°C

d) The likely number of lactobacilli was calculated as follows: The bacterial count on blood agar was subtracted from the bacterial count observed on MRS agar

e) Count of enterococci on SLANETZ´s agar, incubation 2 days at 37°C (SLANETZ and BARTLEY 1957)

f) Count of coliform bacteria on VRB (Violet Red Bile) agar, incubation 1 day at 37°C

g) Count of gram-negative bacteria on ammonium lactate substrate, incubation 2 days at 30°C.

4. Weighing, every second day, for the determination of weight losses.

III. Results

1. Influence of bacterial addition on pH

In the sausages of the control group and in those with M i c r o - c o c c u s addition the pH value went down slowly. During the first two days no decrease at all was observed. In the sausages prepared with L a c t o b a c i l l u s or L a c t o b a c i l - l u s and M i c r o c o c c u s addition the pH was seen to fall rapidly during the first two days, on the average from values around 5.90 to 5.20. The results are presented in Fig. 1.

2. Effect of bacterial addition on organoleptically evaluable qualities

a) Consistency

The consistency developed at about equally slow rate in the sausages of the control group and of the M i c r o c o c c u s group. These characteristics developed much more rapidly in sausages to which lactobacilli had been added. The results are shown in Fig. 2.

b) Color of interior and outer surface

The color of the interior developed slowly in the sausages of the control group. In the sausages with L a c t o b a c i l - l u s addition color defects were frequently noted, consisting of grey or greenish color. The M i c r o c o c c u s addition had a favorable effect on the development of the normal red dry sausage color. Good color developed most rapidly in the sausages to which both micrococci and lactobacilli had been added. This is thought to be due to the reducing effect of the micrococci and to the rapid decrease of pH. The results are seen in Fig. 3.

Similar differences were also observed in the color of the outer surface.

c) Flavor

Fairly raw flavor was often noted in the sausages of the control and M i c r o c o c c u s groups still at 14 days. The M i c r o c o c c u s addition caused only little acceleration of the ripening and the development of normal flavor of dry sausage. The sausages with L a c t o b a c i l l u s addition alone ripened rapidly, but flavor defects were frequently noted. The flavor defect caused by lactobacilli was always the same specific flavor, which is unpleasant when present to a high degree. In the opinion of some of the persons participating in the evaluation, this specific flavor produced a pleasant aroma when slightly present. The sausages with the addition of micrococci and lactobacilli also ripened rapidly. Moreover, flavor defects were never observed in them. The M i c r o - c o c c u s addition has thus been in every instance able to prevent the flavor defects caused by lactobacilli. On the other hand the lactobacilli considerably increased the speed of the ripening process. The results are presented in Fig. 4.

3. <u>Effect of bacterial addition on the age at which the sausages are finished and eatable, and the corresponding weight losses in per cent</u>.

The sausages in the control group were judged to be finished at the age of 19 days on the average. In one test series the sausages of this group were not yet finished at 21 days when the test was discontinued.

M i c r o c o c c u s addition has somewhat accelerated the ripening of the sausages. They were judged to be finished at an average age of 16 days. In one test series the sausages of this group, too, were not yet finished at 21 days. The sausages to which lactobacilli had been added were completed at the age of 12 days on the average. However, three sausages of this group were not finished in 21 days. Distinct flavor and color defects were still observed in them at this time.

The sausages with both L a c t o b a c i l l u s and M i c r o - c o c c u s addition were completed at an average age of 6-7 days. In this group also markedly lower losses of weight noted than in the other groups. The results are presented in Table 1.

Table 1. Average times of completion and corresponding weight losses found on the basis of seven test series.

Sausage group	Average time of completion, days	Corresponding loss of weight, per cent
1. Control group	19	19.8
2. M i c r o c o c c u s group	16	18.3
3. L a c t o b a c i l l u s group	12-13	16.0
4. M i c r o c o c c u s + L a c t o b a c i l l u s group	6-7	6.8

4. Effect of bacterial addition on the microflora of dry sausage.

a) Total bacterial count on blood agar

The bacterial count remained at the highest level in the sausages of the M i c r o c o c c u s group and showed only little decrease during the progress of the test (21 days). A slightly lower level was maintained in the sausages with both L a c t o b a c i l l u s and M i c r o c o c c u s addition. In the sausages of both groups the bacterial flora was mainly composed of the added micrococci.

In the sausages of the control group considerable increase occurred in the bacterial counts during the first seven days, whereupon the count decreased. The bacterial count in the sausages with the addition of lactobacilli alone remained

considerably lower than that in the other groups. The results are seen in Fig. 5.

b) Count of micrococci on blood agar
Micrococci constitute the greater part of the bacteria observed on blood agar, particularly in the sausage types with M i c r o c o c c u s addition. The results are shown in Fig. 6.

c) Total bacterial count on MRS agar
The L a c t o b a c i l l u s count in the sausage mixture was low, on the average some hundreds per gram. Already in sausages of three days' age, however, lactobacilli constituted the major part of the bacterial flora. The bacterial counts on MRS agar attained considerably higher values in the sausages with L a c t o b a c i l l u s addition than in those of the control and M i c r o c o c c u s groups. Fig. 7 shows the results and Fig. 8, the L a c t o b a c i l l u s count.

In the sausages of the control and M i c r o c o c c u s groups the L a c t o b a c i l l u s count was at its highest at 7 days and in those with L a c t o b a c i l l u s addition, at 3 days. According to the results recorded in the study, the addition of micrococci seems to have somewhat lowering effect on the L a c t o b a c i l l u s count.

d) The count of enterococci was usually rather low in the sausage mixture, on the average some hundreds per gram. In the sausages of the control group their count increased on the average to 700.000 bacteria per gram during the first seven days. In all other groups the E n t e r o c o c c u s count remained largely unchanged throughout the test (21 days).

e) The count of gram-negative bacteria fell rather rapidly during the first seven days in all groups except the control group, in which the decrease was slower throughout the ripening period. The rate of decrease was highest in the sausages to which both lactobacilli and micrococci had been added.

f) Also the number of coliform bacteria decreased rapidly in all groups during the first 7-14 days. Only in a few of the sausages coliform bacteria could be observed at the age of seven days or later. The sausages in which coliform bacteria were found at

such a time had been made of a mixture with a coliform considerably higher than normal.

VI. Continued studies

1. Magnitude of Lactobacillus addition required

Some further studies have been carried out in order to find out about the quantity of lactobacilli that has to be added if it is desired to accelerate the manufacturing of dry sausage. The added Lactobacillus quantities were: a) 3-5 million per g, and b) 400,000 per g. Comparison of the sausages manufactured in this manner to the controls in respect of their properties revealed that a Lactobacillus addition of 3-5 million per g is sufficient to cause rapid decrease of pH and to accelerate the manufacturing process. When 400,000 lactobacilli per g of sausage mixture were added, also this addition was found to speed up the manufacturing process. In the lowering of pH no difference was noted during the first three days and a difference was established only after 4-5 days.

2. Experiments with lyophilized L. plantarum strain (No. 4669/6)

The Lactobacillus addition varied between 1 and 15 million per g of the mixture. On comparison of the results with those in the sausages of the control and Micrococcus groups the observation could be made that the manufacturing of dry sausage can be appreciably accelerated also with the aid of a lyophilized Lactobacillus addition. The Micrococcus addition is also now necessary in order to avoid color and flavor defects.

3. Factory experiments

In a meat-processing factory a dry sausage batch of 130 kg was prepared with addition of L. plantarum strain at about 7 million bacteria per g of the mixture and of lyophilized micrococci (Bactofermente) at about 10 million per g of the mixture. Simultaneously, a sausage batch was made with addition of micrococci alone at about

10 million per g. The sausage with added lactobacilli and micrococci was found to become finished in less time than the sausage of the M i c r o c o c c u s group serving as control.

V. Discussion

Our understanding of the contribution of bacteria to the rate at which the ripening process proceeds, to the flavor and to the potential faultiness of the products is still incomplete. Production of sausages often takes place with the aid of a random bacterial flora. It is, therefore, only natural that the products are greatly variable in regard of the quality, flavor and the ripening time. The occurrence of faulty products may also be a frequent consequence. Increased hygiene, for instance, may introduce the drawbacks resulting from an insufficient initial microbial flora. In other factories, again, the initial microbial flora can be unfavorable as a result of poor hygiene.

Some authorities state (ten CATE 1960, KUCHLING 1963) that bacterial additions are not necessary in the manufacture of dry sausage. It would seem natural, however, that also in meat technology attempts would be made to stabilize the ripening process of fermented products by using suitable bacterial cultures. However, such additions have to comply with certain requirements. They should accelerate the rate of completion of the products. The quality of the products should not suffer as their consequence, and under no circumstances should they give rise to faulty products.

In the present study it has been found that L a c t o - b a c i l l u s addition considerably speeds up the ripening of dry sausage. As has also been found by many authorities (e.g. CORETTI 1958, LANG 1960, NIVEN et al. 1949, SKOVGAARD 1963), lactobacilli may cause defective flavor and color in dry sausages. The same was observed in the present study when dry sausage mixture was inoculated with lactobacilli (at 10-40 million per g).

The significance of added micrococci in the production of dry sausage has been criticized (e.g. KUCHLING 1963). However, it has been established in the present study that by means of M i c r o c o c c u s addition it is possible to prevent the said flavor and color defects caused by lactobacilli. Such defects are thought to be due to the oxidative properties of catalase-negative hydrogen peroxide-forming lactobacilli and possibly of streptococci. It seems that this detriment can be counteracted by adding catalase-positive nitrate-reducing micrococci. It was observed also in industrial conditions (NURMI and NIINIVAARA 1964) that color defects of dry sausages can be prevented when a commercial M i c r o c o c c u s preparation is used. The count of nitrate-reducing catalase-positive bacteria in faulty sausages was found to be considerably low. Upon addition of micrococci their count in the finished product was about tenfold.

The experiments reported here have been carried out in a pilot plant where the conditions are not fully equivalent to those in the industry. The quantity of added lactobacilli was comparatively high, though not essentially higher than the recommended quantity of commercial micrococci. It was found out in continued investigations that also with a L a c t o b a c i l l u s quantity of 1-10 million per gram a sufficiently rapid decrease of pH is achieved and the production process can thus be shortened. Good results were also obtained with a lyophilized L. p l a n t a r u m strain. When the same L a c t o - b a c i l l u s strain was used in industry as a meat broth culture, the processing time could be appreciably shortened.

Comments on the effect of lactobacilli on the development of flavor in dry sausages have been presented e.g. by LERCHE (1955). It has been found in the present studies that in the opinion of many people the specific flavor caused by lactobacilli, when present in slight degree, gives a pleasant extra aroma to the sausage.

SUMMARY

In studies concerning the effect of various bacterial additions on the properties of dry sausage the following observations could be made:

1. L a c t o b a c i l l u s addition causes rapid decrease of pH, considerably accelerating the ripening time of the sausage. In many instances, however, lactobacilli produce defective flavor and color of the sausage.

2. M i c r o c o c c u s addition does not very substantially speed the decrease of pH, nor does it accelerate the ripening time. However, this addition possesses remarkable significance in that it prevents the flavor and color defects caused by lactobacilli.

3. By simultaneous use of L a c t o b a c i l l u s and M i c r o c o c c u s addition the manufacturing time of dry sausage could be shortened on an average from 19 to 6-7 days in the experimental conditions of the present study. Correspondingly, the weight losses went down from about 20 to about 7 %.

4. L a c t o b a c i l l u s and M i c r o c o c c u s addition exerts an inhibitory effect on the non-desired bacterial flora in dry sausage.

ZUSAMMENFASSUNG

Beim Untersuchen des Einflusses verschiedener Bakterienzusätze auf die Eigenschaften der Rohwurst konnte folgendes festgestellt werden:

1. L a c t o b a c i l l u s zusatz bewirkt einen raschen Abfall des pH-Werts und verkürzt beträchtlich die Reifungzeit der Wurst. In zahlreichen Fällen verursachen Lactobazillen jedoch Geschmacks- und Farbfehler in den Würsten.

2. M i c r o c o c c u s zusatz beschleunigt nicht sehr wesentlich den Herabgang des pH-Werts und auch nicht die Reifungzeit. Dieser Zusatz besitzt jedoch merkliche Bedeutung, indem er die von Lactobazillen hervorgerufenen Fehler in Geschmack und Farbe verhindert.

3. Durch gleichzeitige Anwendung von L a c t o b a c i l l u s - und M i c r o c o c c u s zusatz konnte in den vorliegenden Versuchsverhältnissen die Fertigungszeit der Rohwurst im Durchschnitt von 19 auf 6-7 Tage verkürzt werden. Entsprechendermassen gingen die Gewichtsverluste von etwa 20 auf etwa 7 % herab.

4. L a c t o b a c i l l u s - und M i c r o c o c c u s zusatz wirken inhibierend auf die unerwünschte Bakterienflora in der Rohwurst ein.

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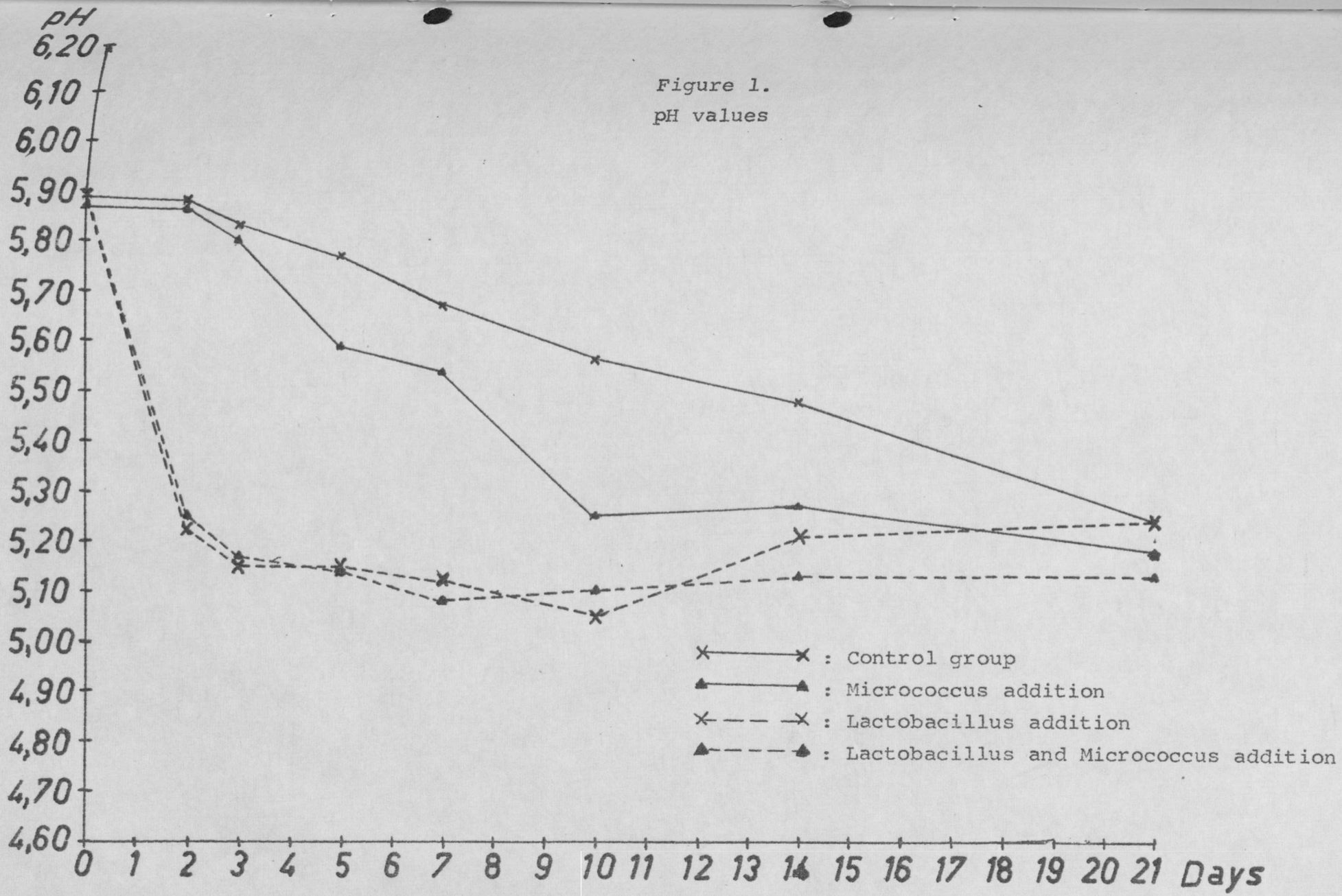


Figure 1.
pH values

Figure 2.
Consistency

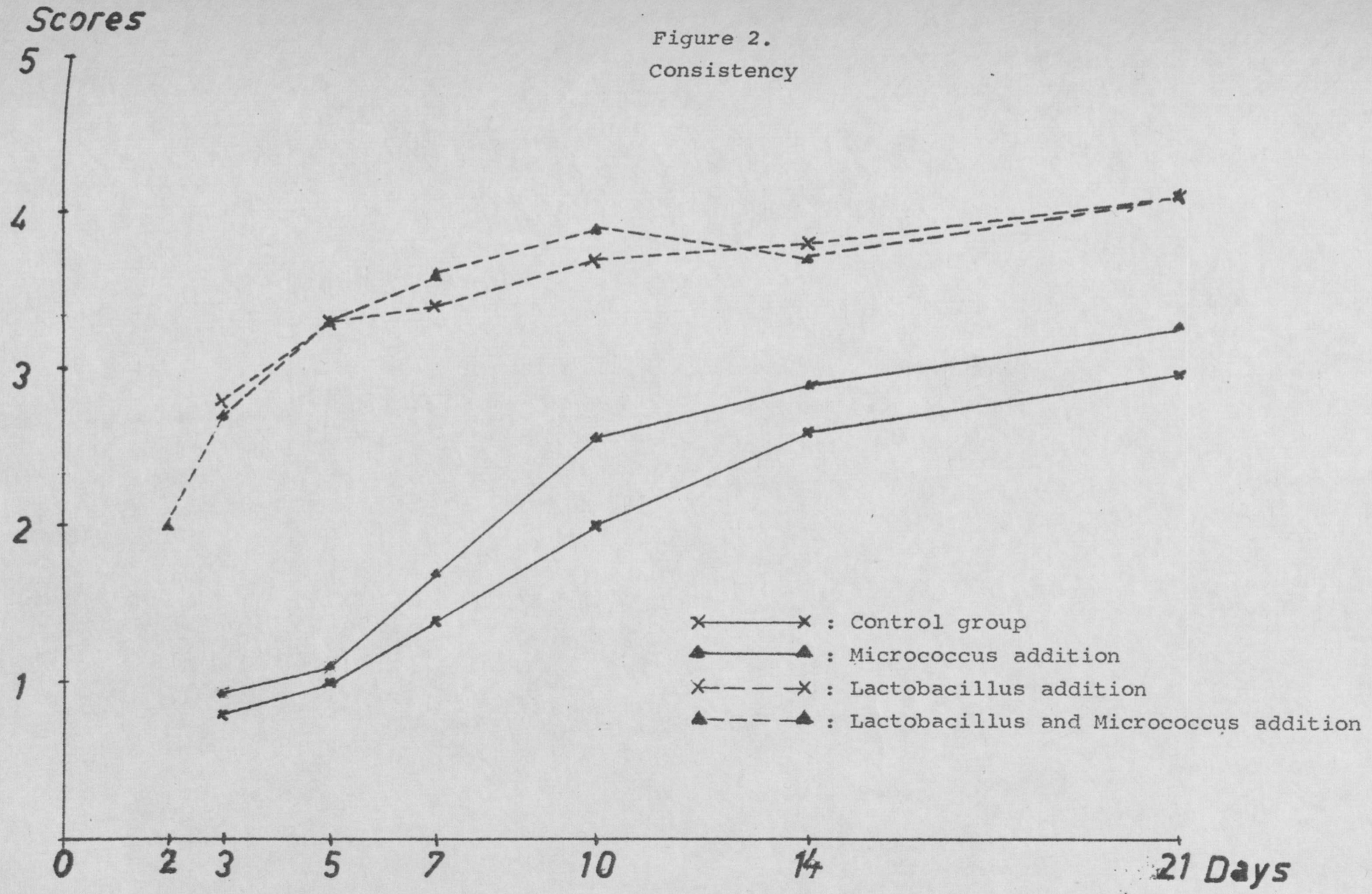


Figure 3.
Color of inner surface

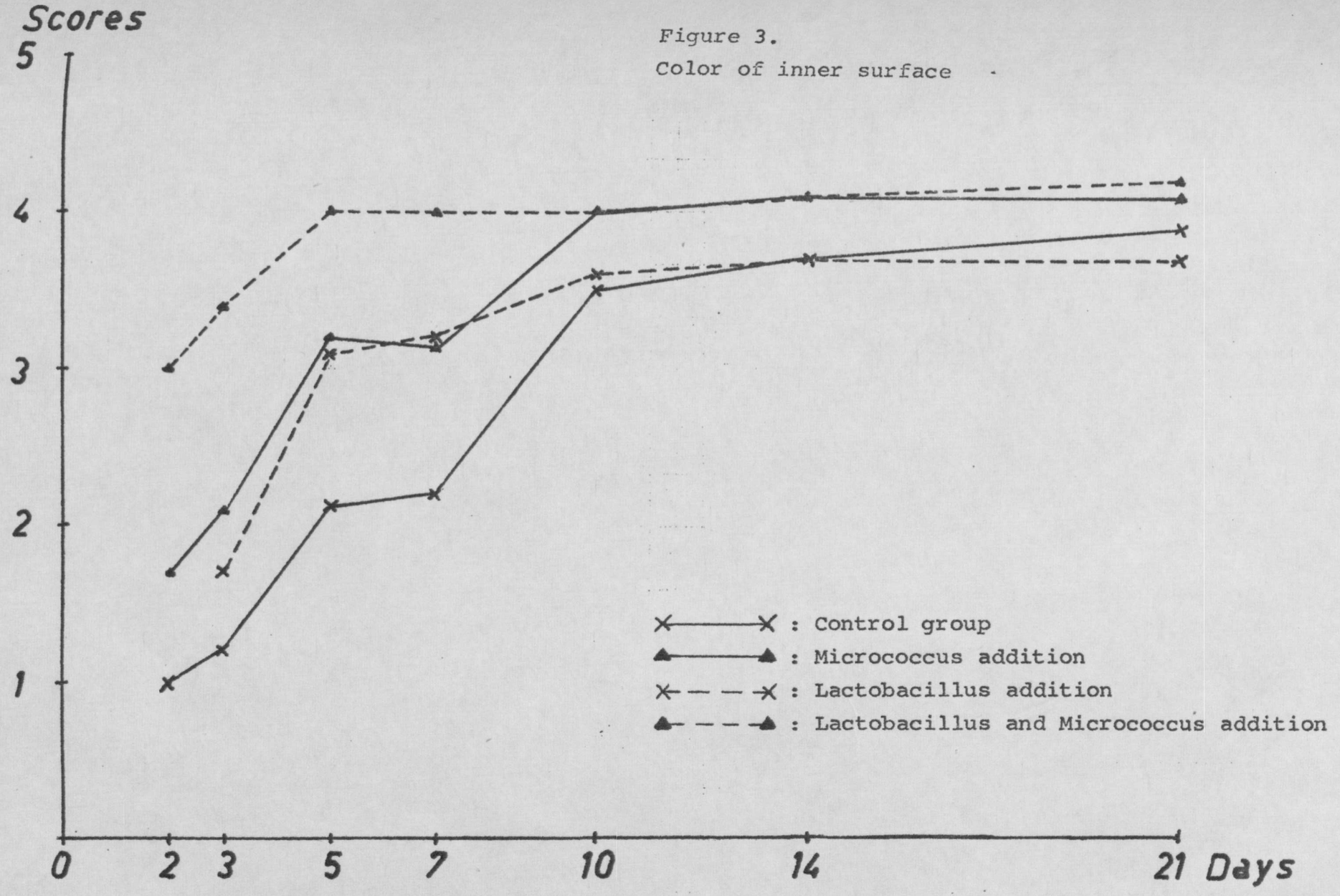


Figure 4.
Flavor

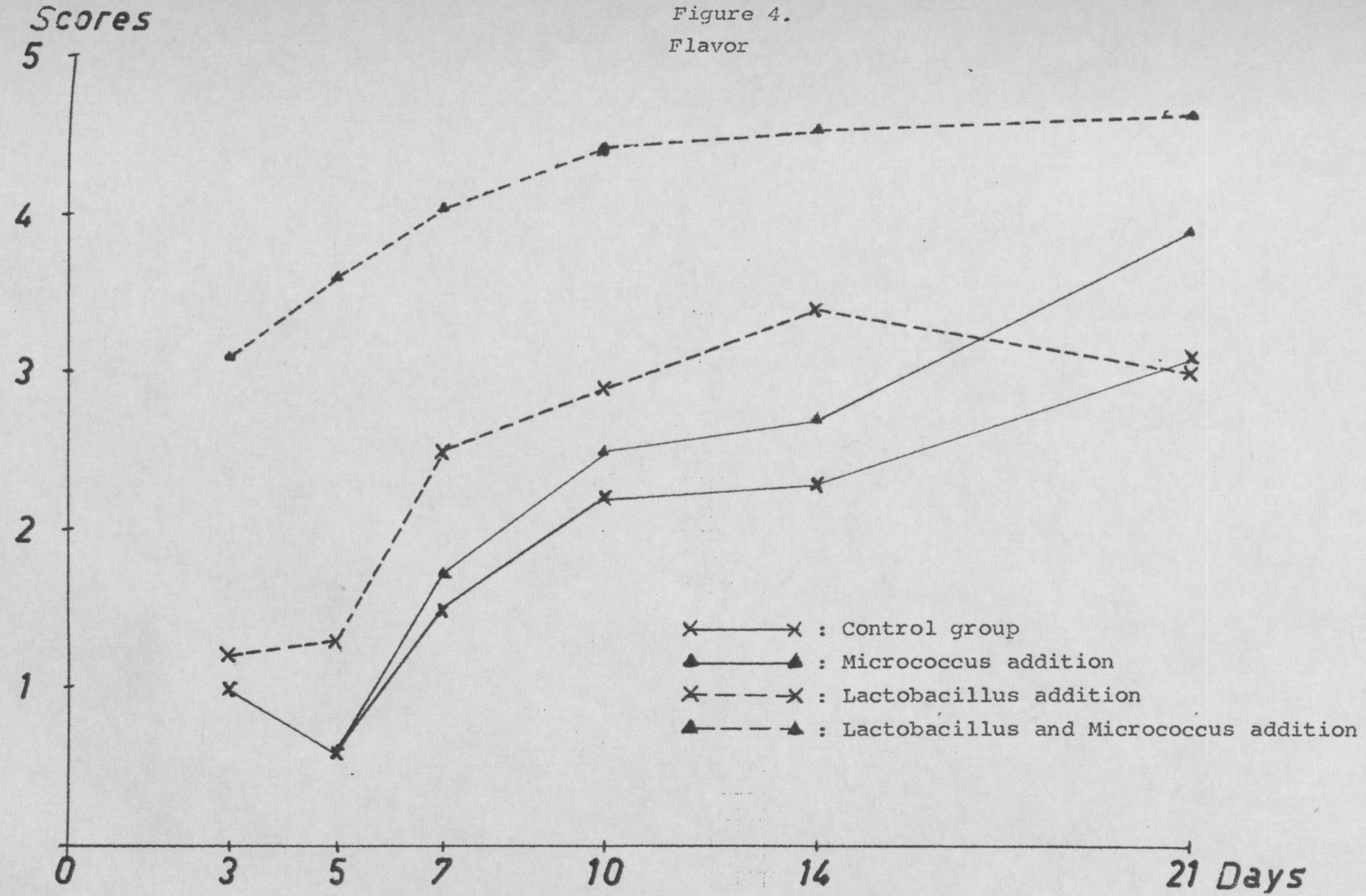
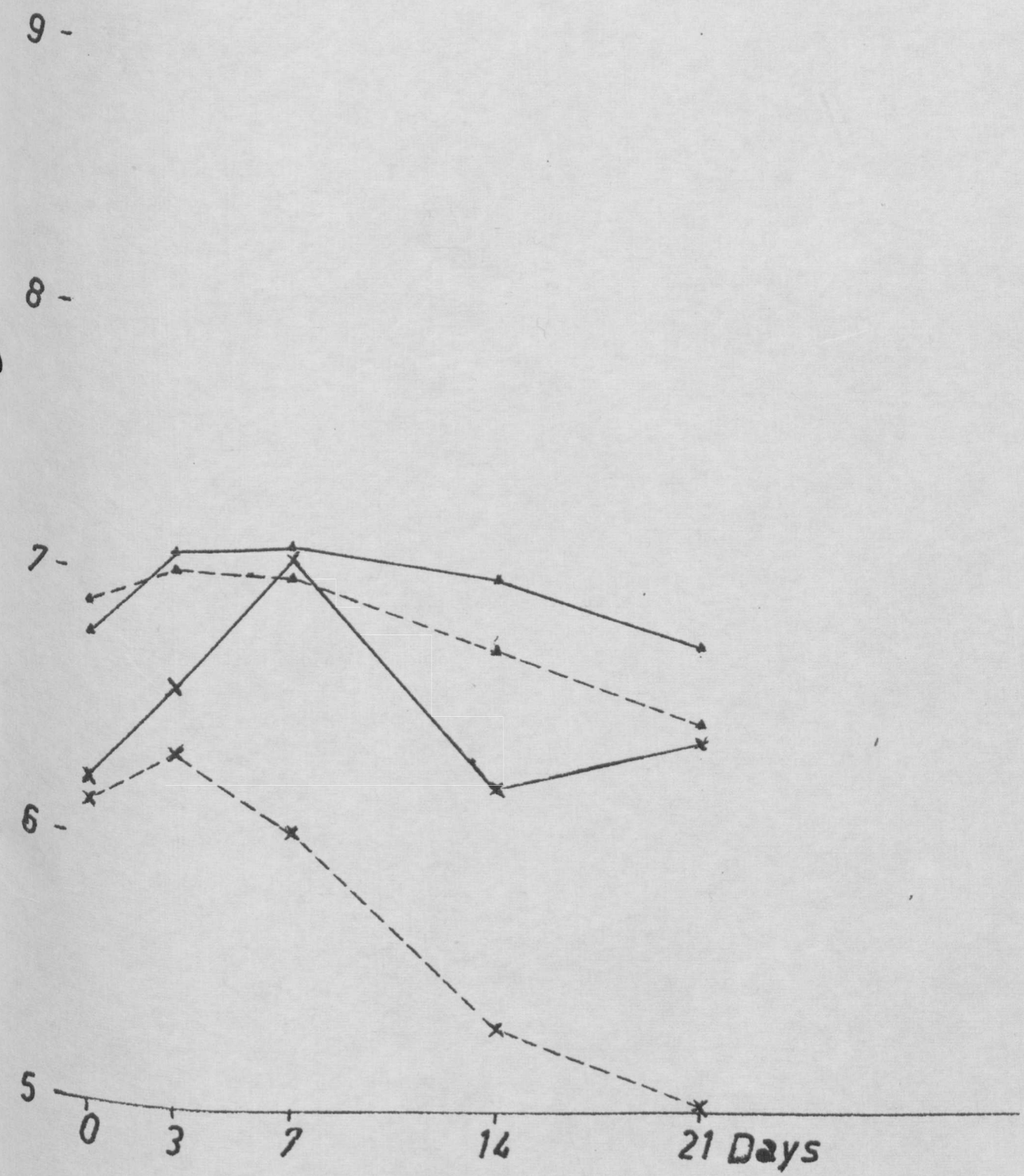
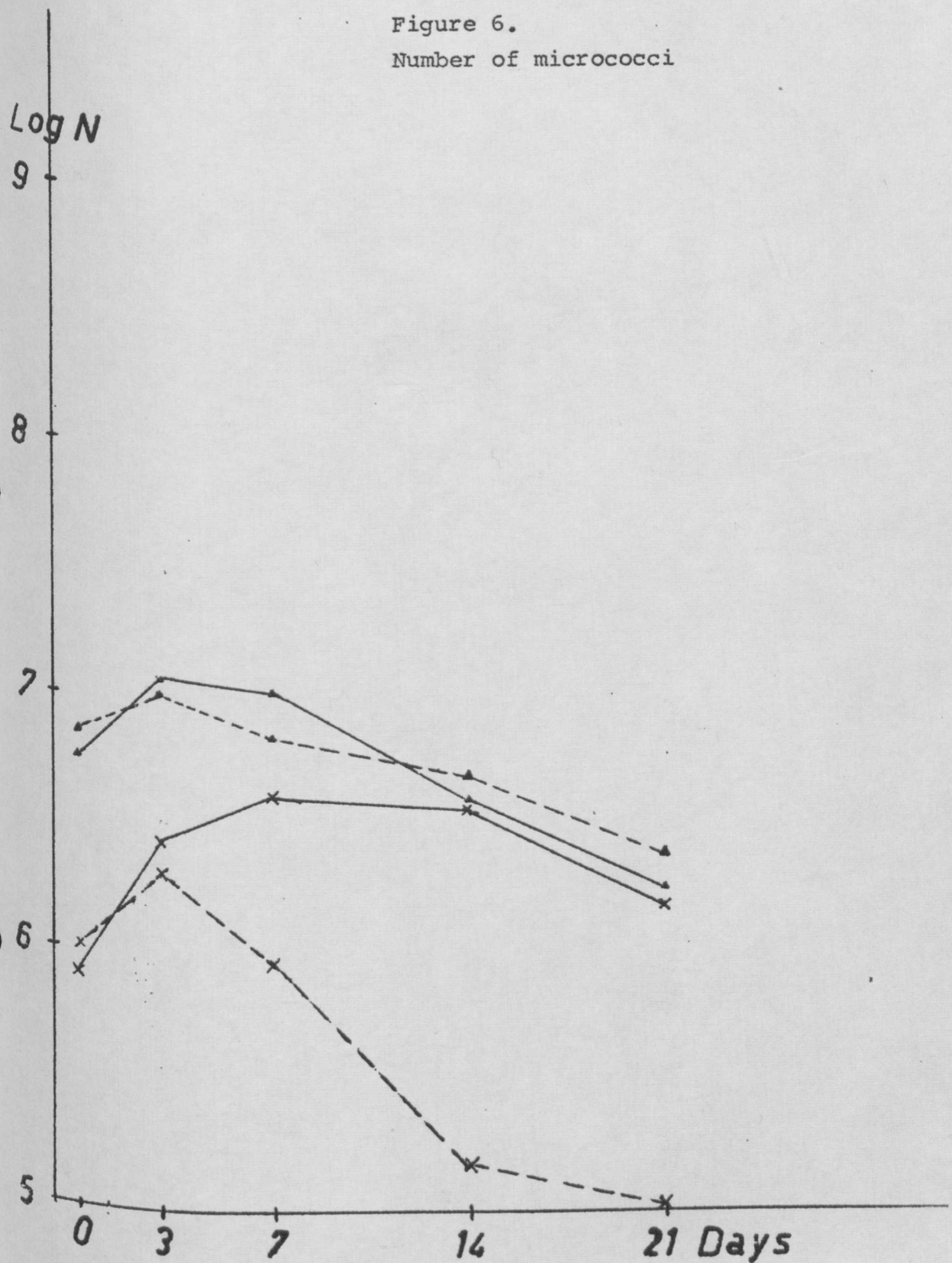


Figure 5.
Number of bacteria on blood agar



: Control group
: Micrococcus addition
: Lactobacillus addition
: Lactobacillus and Micrococcus addition

Figure 6.
Number of micrococci



: Control group
: Micrococcus addition
: Lactobacillus addition
: Lactobacillus and Micrococcus addition

Figure 7.
Number of bacteria on MRS agar

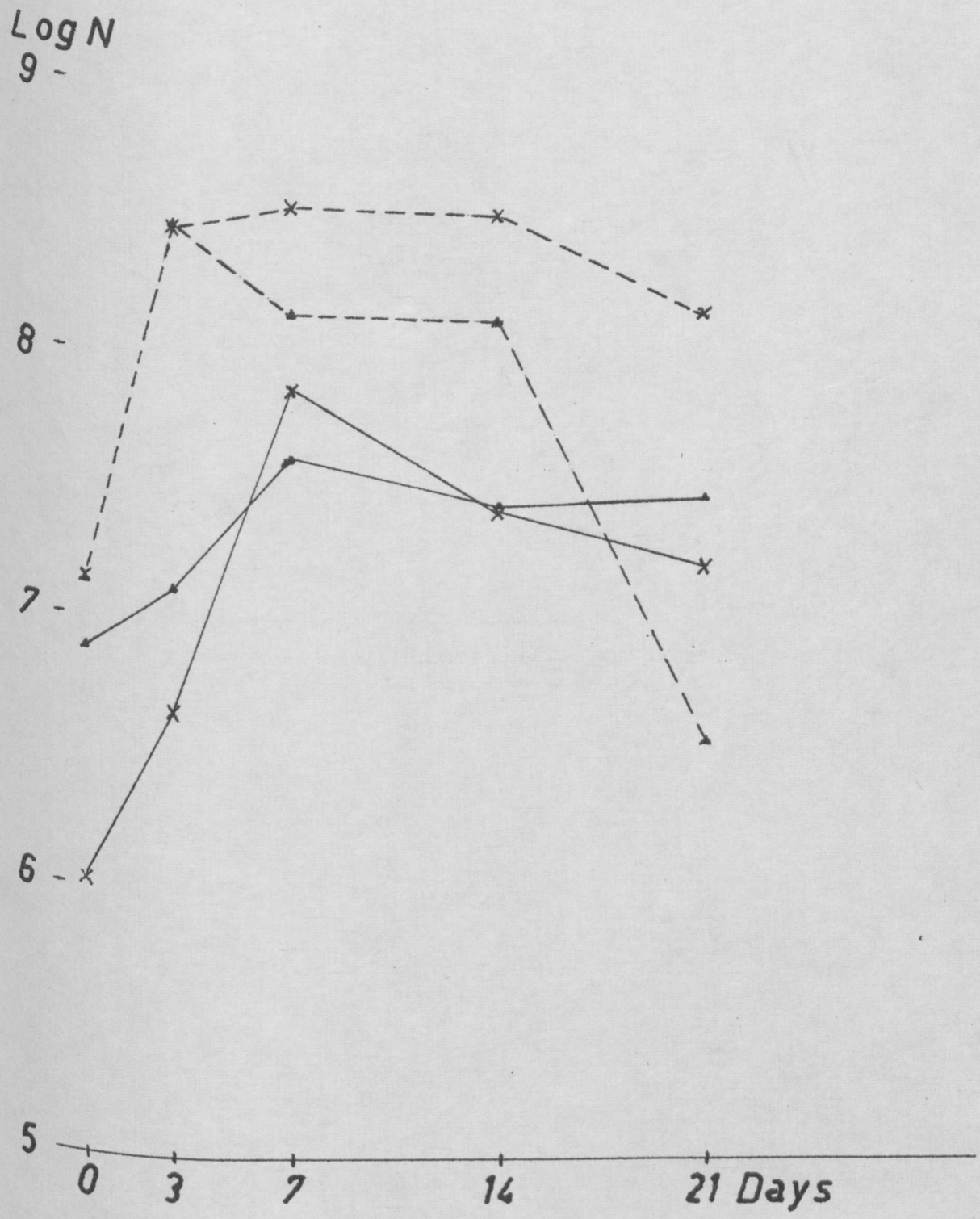


Figure 8.
Number of lactobacilli