7:14 # A study of commercial fermented sausages production using natural fermentation, starter cultures and glucono-delta-lactone

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Introduction

Because dry fermented sausages do not receive heat treatment during processing and their production is based on the proper development of usefull microorganisms a successful fermentation is essential for a good quality and wholesome product.

The original method of fermented sausage production, which is still in use today, is based upon chance inoculation or on the use of "sausage" starter. In the second case finished salami, from previous runs, is added to the meat mixture as a source of the desirable microorganisms. Natural fermentation with chance inoculation although giving a satisfactory product it may lead to a poor quality product with considerable economic losses (11, 26). Also with the use of sausage starter there is uncertainty regarding the type, number and activity of the microorganisms added to the meat mixture (12, 14).

The employment of starter cultures, which was introduced in the 1950's, allowed the addition of the desirable specific type and number of microorganisms and permited the regulation of the direction and speed of the fermentation. Several investigators discussed the benefits of starter cultures (5, 9, 13, 14, 17, 29).

The use of glucono-delta-lactone which originally was introduced in the 1960's in the cooked comminuted products for rapid color development later found application to the fermented sausage production for rapid acidification (1, 2, 17).

Traditionally the industrial production of fermented salami sausages in Greece is based on natural fermentation and/or on the use of natural "sausage" starter. However recently some modern industries have started experimental production using pure starter cultures or addition of glucono-delta-lactone. The purpose of the present research was to study the relative effects on commercially produced fermented salami using a) natural fermentation,b) sausage starter from previous runs c) commercial mixed culture of lactobacilli and micrococci, d) commercial pure culture of micrococci and e) 0.4% glucono-delta-lactone.

Materials and Methods

Sausage formulation and processing. All five treatments were reproduced three times in a modern sausage factory, located in the Thessaloniki area of Northern Greece, during the course of its normal daily production, utilizing the same raw material and ingredients and applying the same processing conditions of fermentation and ripening.

The five treatments of this study were:

A Natural fermentation

B Sausage starter culture (750 g/100 Kg meat mixture) from ground salami (30 days old) from previous runs

C Commercial freeze dried mixed culture of lactobacilli (L.plantarum) and micrococci (M.violagabriella) added at a level 50 g/100 Kg meat mixture (Compi Start 1505*)

D Commercial freeze dried culture of micrococci (M.violagabriella) added at a level 25 g/100 Kg meat mixture (Micro Start 10*)

E Glucono-delta-lactone** (400 g/100 Kg meat mixture).

The composition of the meat mixture for all treatments was: 23% beef, 34% pork, 10% mutton and 33% pork back fat (lard). The common ingredients added to the meat mixture, per 100 Kg of it, were: 3% salt, 0.05% (500 ppm) sodium nitrate, 0.02% (200 ppm) sodium nitrite, 0.5% sugars, 0.05% sodium ascorbate, 0.4% white pepper and 0.2% garlic.

Prior to use the imported frozen boneless beef, pork and mutton meats, which were stored at -18°C, were thawed at 4°C. The lard was kept at -12°C until use. The thawed meats and frozen lard were pre-cut in large pieces and separated in appropriate weights. The preweighted amounts of beef, pork and mutton were chopped in cutter and the dry ingredients and starter cultures were dispersed in the mixture. Finally the frozen pieces of lard were added and the mixture was cut to the desired particle size of the product. After preparation the sausage mixture had the following average composition: moisture 48.60%, fat 30.70% non-fat organic matter 17.20% and ash 3.50%.

The prepared sausage mixture, with the aid of a vacuum stuffer, was stuffed into 60 mm diameter fibrous casings to make standard pieces of 1.5-2 Kg each. The resultant strings of sausages were placed for 7 days in the fermentation room under the following conditions: starting temperature 23°C progressively reduced to 18°C, starting relative humidity 94% reduced progressively to 82%, air movement 0.5 to 0.7 m/sec and complese dark. During the 2nd or 3rd day of fermentation the sausages were smoked. On the 7th day the strings of sausages were transferred to the ripening room where they were kept for 3 to 4 weeks under the following conditions: temperature between 13° to 15°C, relative humidity from 70 to 80%, air movement 0.05 to 0.1 m/sec and complete dark.

Individual sausages from each treatment were taken at 0, 1, 4, 7, 14, 21 and 28 days after preparation. The samples were placed in a portable refrigerator and transported to the laboratory within 1 hour. Upon receipt the samples were analysed the same day.

Microbiological analyses. For microbiological analysis the sausages were sliced into two aproximately equal parts and samples were taken from cross sections on either side of the center (20). Twenty grams of each sample together with 180 ml of sterile 0.1% peptone (BBL) water were added to a sterile Waring Blendor jar and blended for 2 minutes at high speed. The resulting slurry with appropriate dilutions was used for the determination of total aerobic bacteria, lactobacilli (Gram (+), catalase (-) rods), micrococci and non-pathogenic staphylococci (Gram (+), catalase (+) cocci), enterobacteria (Gram (-) rods), Staphylococcus aureus and yeasts. Methods, media and incubation conditions used are presented in Table 1. Gram stain and catalase test were performed on the colony types grown on the media. Coagulase test (4) was performed for S.aureus colonies with doubtful characteristics. Counts obtained were recorded as log of bacteria cells per gram of sausage.

Physical measurements. Sausage color was measured with a Tru-color Neotec Instruments meter which was standardized against a standard plate (L=+96, a=-1.03 and b=+2.4). For the measurements thin slices (3-5 mm thick) were used which were aligned to cover the entire area of the meter window (7).

The firmness, expressed in shore units, was measured at the core of the sausage using a Zwick consistometer, as recommended by Linhard and Lieppe (18).

Chemical analyses. The remaining sausage, after microbiological assays and color measurements, was twice ground in a Kenwood mincer (1/8 plate) and used for chemical analyses.

Moisture, fat and ash content were determined by standard AOAC procedures (3). For pH determination 20 g of sausage were blended with 180 ml of distilled water for 30 sec in a Waring Blendor and the pH of the resulting slurry was measured with a Beckman digital pH meter (8). Afterward the slurry was filtered through a Whatman N° 42 filter paper and the titratable acidity of the filtrate was measured with 0.1 N NaOH using phenolphthalein as indicator. The titratable acidity was calculated as lactic acid % (4, 16).

Water activity (a_w) was determined using a Nova-Sina (Model E.E.J-3, Switzerland) water activity instrument. The measurements were obtained after an equilibration period of 2 hours at 20°C.

Table 1. Methods, media and incubation conditions used for microbiological analyses

Microorganisms	Method	Media	Incubation	References
1.Total aerobic bacteria	Plating	APT agar (Merck)	25°C 4 days	20, 23
2.Lactobacilli	Plating 2nd layer	Rogosa SL agar (Merck)	25°C 4 days	20, 23, 27
3.Micrococci and Staphylococci	Spreader	Mannitol salt agar (Difco)	37°C 2 days	20, 27
4.Enterobacteria	Plating 2nd layer	VRBG agar (Merck)	37°C 24 h	15, 31
5.S.aureus	Spreader	Baird-Parker agar (Merck)	37°C 48 h	4
6.Yeasts	Plating	Potato Dextrose agar (Merck)	25°C 3 days	4, 23

Sensory evaluation. A panel consisting of 25 plant and laboratory members was used to evaluate the 28 days old sausages for color, flavor and overall acceptability with an eight point hedonic scale (1=dislike extremely, 8=like extremely). Panelists were served two slices of sausage (3 mm thick) from each treatment along with unsalted crackers and a glass of water. The slices of each treatment were randomly placed on the disc. The panelists were instructed to evaluate each sample after rinsing their mouth with water (7).

Statistical analysis. Data from physicochemical measurements and results from sensory evaluation were statistically treated with analysis of variance and the significance of means was tested using Duncan's new multiple range test (30).

Results and Discussion

Microbiological examination. The growth of microorganisms during processing is shown in Fig 1.

The total plate count on zero day (day of formulation) was lower in the treatments using natural fermentation (5.86) and GdL (6.07) and higher in the treatments using sausage starter (6.90) and culture of micrococci (6.93). Considerably higher (8.02) was the total count in the treatment using mixed culture of lactobacilli and micrococci. The first day of fermentation the total count remained the same whereas between the 1st and 4th day a considerable increase was observed in all treatments. On the 4th day the total count ranged between 8.20 and 8.84 the lowest value corresponding to the GdL treatment. After the 4th day the count remained almost constant and ranged at the same level in all treatments.

A similar pattern was obtained with lactobacilli. On zero day the count of lactobacilli in the treatments using natural fermentation, GdL and culture of micrococci ranged between 3.61 and 4.33 while their count was considerably higher in the treatments using sausage starter (5.58) and mixed culture of lactobacilli and micrococci (6.18). The first day of fermentation the count of lactobacilli showed a slight increase in all treatments. Between the 1st and 4th day the count of lactobacilli showed a rapid increase reaching on the 4th day counts between 8.32 and 8.83. The highest count was observed in the treatments using sausage starter and mixed culture of lactobacilli and micrococci. After the 4th day the count of lactobacilli remained constant in all treatments. These data agree with the results of Skjelkvale et al (28).

The count of micrococci and non-pathogenic staphylococci the zero day ranged between 4.45 and 6.25. The highest count (6.25) was found in the treatment using culture of micrococci. The count of micrococci and staphylococci showed the same pattern in all treatments. It increased the first 4 days and then showed a small decrease up to the end of fermentation. During ripening the count remained almost constant and on the 28th day the count of micrococci and staphylococci ranged between 5.80 and 6.15.

The yeast counts showed a similar pattern in all treatments. On zero day the count of yeasts ranged between 3.25 and 4.07. The count increased during fermentation and during ripening a small decrease was observed. On the 28th day the yeast count ranged between 3.90 and 4.30.

These findings show that, except for the day of formulation (zero day), the counts of aerobic bacteria (total plate count), of lactobacilli, of micrococci and staphylococci as well as the count of yeasts ranged at the same levels and showed the same pattern in all treatments.

Considerable differences among the various treatments were observed with the counts of enterobacteria and S.aureus. The count of enterobacteria in all treatments showed a continous decrease during fermentation and ripening. The treatment using natural fermentation had a considerably higher count on zero day (4.12) which remained higher than the other treatments throughout processing. High counts of enterobacteria were also shown in the treatments using sausage starter and culture of micrococci. On the contrary the treatments using GdL and mixed culture of lactobacilli and micrococci had a considerably lower count (2.67-2.84) which continuously decreased and disappeared the 21st day. The initial count of S.aureus (zero day) ranged between 2.17 and 2.84. In the treatments using natural fermentation, sausage starter and culture of micrococci it increased the first 4 days of fermentation and then showed a continuous decline. The treatment using natural fermentation had the highest count of S.aureus throughout fermentation and ripening. The 4th day it was 3.95 and the 28th day had dropped to 2.00. In the treatments using GdL and mixed culture of lactobacilli and micrococci the S.aureus count showed a continuous decrease and disappeared the 28th day. The inhibitory action of the mixed starter culture and of the GdL on the growth of S.aureus is due to the rapid fall of the pH caused by GdL hydrolysis and/or the large number of lactic acid bacteria which accelerate the rate of acid production. It is also due to the antagonistic effects of the mixed starter cultures on the staphylococcal growth (5, 10, 22).

Physicochemical parameters. Observed differences during fermentation and ripening in pH, total acidity and firmness are shown in Fig.2.

The mean initial pH values on zero day of the five treatments ranged between 6.23 and 6.25. The treatment using GdL had an initial pH of 5.7. This considerable drop of the pH was due to the rapid hydrolysis of GdL and it is in agreement with the findings of other investigators (1, 21). Between the 1st and 4th day of fermentation a considerable drop in the pH was observed in all treatments. The rate of the pH decrease was greater in the treatment using mixed culture (pH 5.14) and smaller in the treatments using natural fermentation (pH 5.69) and GdL (pH 5.30). The decrease in pH continued until the end of fermentation (7th day) in the treatments using mixed culture and sausage starter whereas in the treatments with natural fermentation and pure culture of micrococci and GdL the decrease continued at a very slow rate up to 14th day. During the last two weeks of ripening the pH remained almost constant in the treatments with

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mixed culture and with GdL while a small increase in the pH was observed in the treatments with natural fermentation, sausage starter and pure culture of micrococci. The small increase in pH at the beginning of fermentation and at the end of the ripening period is attributed to the action of proteolytic bacteria (14, 19, 24). The treatment with natural fermentation showed the highest mean value of pH during processing while the treatments using mixed culture of lactobacilli and micrococci showed the smallest mean value of pH. In the final product the pH ranged between 5.02 and 5.32 and there were no significant differences ($P<0.05$) among treatments. Commercial Greek fermented salami sausages were found to have a pH ranging between 4.57 and 5.03 (25).

The total acidity, expressed as lactic acid %, showed the same pattern in all treatments and it was almost inversely proportional to the observed pH decrease. On zero day the total acidity ranged between 0.31% and 0.40%. A small decrease was observed the first day of fermentation. This decrease in lactic acid, which corresponded to a slight increase in pH, was also observed by List and Klettner (19) and Joseph et al (16). Afterward a rapid increase continued up to the 14th day after which the total acidity increased at a slower rate up to the 28th day. The treatment using natural fermentation showed the lowest total acidity during fermentation and ripening whereas the highest total acidity was shown by the treatments using GdL and mixed culture of lactobacilli and micrococci. In the final product the total acidity ranged between 0.55% and 0.73%. The final total acidity of the treatment with natural fermentation was found significantly different ($P > 0.05$) from the other treatments.

In all treatments the firmness, at the core of sausage, showed a continuous increase during fermentation and ripening. On the 1st day of fermentation a slight increase in firmness was observed in the treatments using GdL, sausage starter and mixed culture of lactobacilli and micrococci whereas in the other treatments no change in consistency was observed. After the first day of fermentation, a continuous increace in firmness was observed in all treatments. The highest rate of firmness increase was observed during the first days of ripening. The treatment using natural fermentation showed the least firmness during processing which was significantly different ($P > 0.05$) from the other treatments except the treatment with sausage starter. The least firmness (soft texture) observed in the treatment with natural fermentation is related to the higher pH shown by this treatment and it is in agreement with the observations of Deibel et al (11) . The treatment with GdL showed the highest firmness during processing followed, without significant difference ($P<0.05$),by the treatment using mixed culture of lactobacilli and micrococci.

Values of moisture content and water activity (a_w) during fermentation and ripening are shown in Fig. 3.

On zero day the moisture content of the treatments ranged between 47.12% and 48.72% with no significant difference ($P < 0.05$) among treatments. During the first days of fermentation there was a slight increase in moisture content in all treatments except the treatment with GdL. This slight increase may be due to the absorption of moisture from the environment. After the 4th day the moisture content in all five treatments decreased continuously until the 28th day when it ranged from 31.51% to 33.23% with no significant difference ($P<0.05$).

The water activity on zero day ranged from 0.957 to 0.960. The first days of fermentation a slight increase of a_w occured in all treatments except the GdL treatment which corresponds with the observed moisture increase. After the 4th day the a_w continuously decreased until the end of ripening. The smallest value of a_w during ripening was in the treatment with mixed culture of lactobacilli and micrococci and the highest value of a_w was in the treatment using natural fermentation. The average a_w at the end of ripening ranged between 0.902 and 0.909 with no significant difference ($P < 0.05$) among treatments.

The correlation coefficients of the above measured parameters are given in Table 2.

Table 2. Correlation coefficients among measured physicochemical parameters during fermented sausages production

	pH	Total acidity (lactic acid %)	Firmness	Moisture content %
Total acidity (lactic acid %)	-0.910			
Firmness	-0.705	0.904		
Moisture content (%)	0.625	-0.838	-0.972	
Water activity (a_w)	0.594	-0.816	-0.970	0.981

The highest correlation (r=0.981) was found between a_w and moisture.The next highest (r=0.972) between moisture and firmness followed by a_w and firmness (r=0.970) and total acidity and pH (r=-0.910). Lower coefficients were found between firmness and total acidity (r=+0.904), moisture content and total acidity (r=0.838), a_w and total acidity (r=-0.816) and between firmness and pH (r=-0.705). The lowest correlation coefficients occured between moisture content and pH (r=+0.625) and between a_w and pH (r=+0.594). These results are in complete agreement with the findings of Baumgartner et al (6).

Color measurement. Values of color a(+) (redness), L (lightness) and b(+) (yellowness) are shown in Fig 4.

The a(+) value on zero day ranged between 7.30 and 9.70 and the treatment with GdL showed the highest significantly different ($P > 0.05$) value. The first day of fermentation the a(+) value showed an almost vertical increase to a range from 20.35 to 21.51. This rapid increase in redness development due to the formation of cured pigment is in accordance with the findings of Zaika et al (32). From the 1st to the 7th day there was a decrease in a (+) value which can be attributed to the dissociation of nitric oxide heme pigment and moisture absorption . After the 7th day until the end of ripening the a(+) values were stable without significant difference ($P<0.05$) among the treatments. The L value at zero day ranged between 49.71 and 51.22. During the first days of fermentation showed a considerable decrease and between the 4th and 7th day the L value increased sharply after which there was a continuous decrease. No significant differences ($P<0.05$) were found among the treatments. The b(+) values showed a continuous decrease during fermentation and remained almost constant during ripening. No significant differences ($P<0.05$) were found among the treatments.

Sensory evaluation. Results from organoleptic evaluation of the final products are presented in Table 3.

Color. There were no significant differences ($P<0.05$) in color evaluation between the five treatments in any of the three production lots. Color scores ranged between 6.04±1.48 and 6.92±0.95 in a scale of 1 to 8 (8=like extremely). The highest score in color was observed in the treatment using sausage starter.

Flavor. The treatment using mixed culture of lactobacilli and micrococci showed the highest score in all three production lots ranging between 5.96±1.16 and 6.24±1.64. The treatment using culture of micrococci showed the lowest score (4.84±1.90) in one of the three production lots which was significantly different ($P > 0.05$) from the other treatments.

Overall acceptability. The highest scores in all three production lots were found in the treatments using mixed culture of lactobacilli and micrococci (5.90±1.56 - 6.40±1.04) and GdL (5.90±1.03 - 6.34±0.91).

Table 3. Results from sensory evaluation on the 28th day

Treatments	Color* 1st	Color* 2nd	Color* 3nd	Flavor* 1st	Flavor* 2nd	Flavor* 3nd	Overall acceptability* 1st	Overall acceptability* 2nd	Overall acceptability* 3nd
A	6.60^{a} ±1.11	6.04^{a} ±1.48	6.38^{a} ±1.41	5.96^{a} ±1.51	5.76^{a} ±1.26	5.73^{a} ±1.30	5.60^{a} ±1.68	5.84^{a} ±1.31	5.96^{a} ±0.86
B	6.92^{a} ±0.95	6.32^{a} ±1.31	6.50^{a} ±1.22	5.90^{a} ±1.59	5.84^{a} ±1.17	5.70^{a} ±1.41	5.84^{a} ±1.65	6.00^{a} ±0.91	5.78^{a} ±1.24
C	6.60^{a} ±0.86	6.08^{a} ±1.15	6.40^{a} ±1.02	6.24^{a} ±1.64	6.20^{a} ±1.19	5.96^{a} ±1.16	5.96^{a} ±1.59	6.40^{a} ±1.04	5.90^{a} ±1.56
D	6.44^{a} ±1.41	6.10^{a} ±1.22	6.13^{a} ±1.19	5.88^{a} ±1.53	4.84^{b} ±1.90	5.90^{a} ±1.68	5.88^{a} ±1.53	4.92^{b} ±1.68	5.83^{a} ±1.17
E	6.88^{a} ±1.16	6.12^{a} ±1.11	6.46^{a} ±1.53	6.04^{a} ±1.30	6.16^{a} ±1.81	5.80^{a} ±1.07	6.00^{a} ±1.42	6.34^{a} ±0.91	5.90^{a} ±1.03

* Based on an 8 point hedonic scale where 1=dislike extremely and 8=like extremely. 1st, 2nd, 3nd:indicate production lot
a, b Means in the same column with the same superscripts are not significantly different ($P<0.05$)

The treatment using pure culture of micrococci showed in one of the three production lots the smallest score (4.92±1.68) which was significantly different ($P>0.05$) from the scores of the other treatments. Results indicate that flavor rather than color had the greater influence on the overall acceptability of the product.

Conclusions

The treatment using natural fermentation gave a good product with a satisfactory color and flavor score. However the rate of pH decrease was slower and the final pH was higher than in the other treatments. The product had the lowest total acidity, the least firmness and the highest count of enterobacteria and S.aureus.

The treatment with sausage starter also gave a good product with the highest score in color and a satisfactory flavor score. In addition it showed a faster rate of pH decrease, higher acidity and better firmness than in the natural fermentation treatment. However the count of enterobacteria and S.aureus were only slightly lower than the treatment with natural fermentation.

The treatment using mixed starter culture of lactobacilli and micrococci gave a final product with the highest score in flavor and a satisfactory color score. The rate of pH decrease was faster than all the other treatments. The total acidity and firmness were satisfactory and counts of enterobacteria and S.aureus were maintained at very low level during processing. Based on these results it seems that the use of a mixed culture of lactobacilli and micrococci offers a certain advantage for increasing the uniformity and safety as well as improving the product quality.

The treatment using pure culture of micrococci although giving a good quality product in two production lots, failed to give a product with satisfactory flavor in one of the three production lots. However the rate of pH decrease was better than with natural fermentation and the total acidity was higher and the firmness more satisfactory than the treatment using sausage starter. Also the count of enterobacteria and S.aureus remained at lower levels than the above two treatments.

Addition of 0.4% glucono-delta-lactone gave a final product comparable to that obtained with the use of mixed culture of lactobacilli and micrococci. The fast rate of pH decrease and the low pH aided the product to obtain the highest total acidity and firmness, with a satisfactory color and flavor, while the counts of enterobacteria and S.aureus were mantained at the lowest level.

No significant differences ($P<0.05$) were observed among the treatments during fermentation and ripening in total count, lactobacilli, micrococci and staphylococci, yeasts count or in moisture content, water activity and color measurements.

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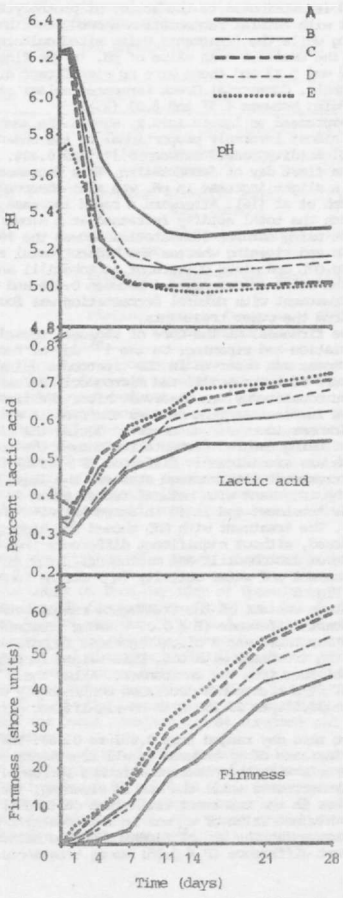

Fig. 2. Changes in pH, total acidity (lactic acid %) and firmness (based on three commercial lots)

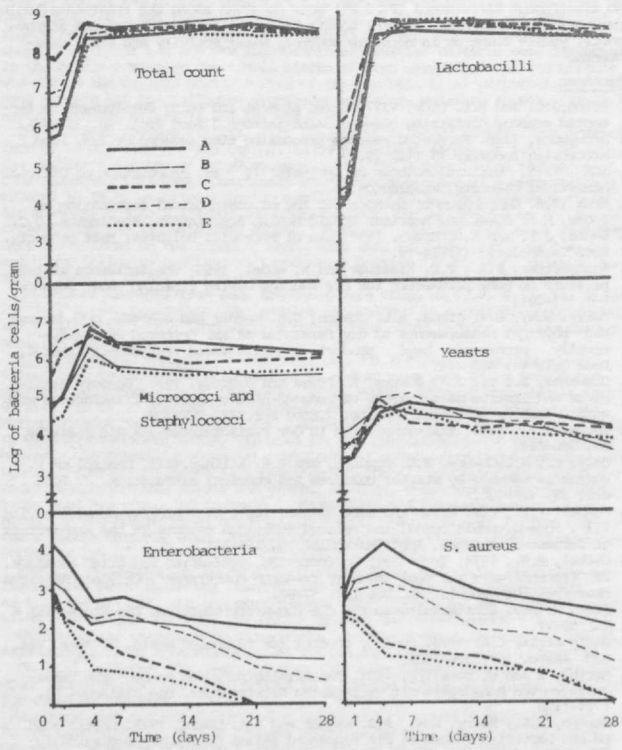

Fig.1. Growth of microorganisms during processing (based on three commercial lots)

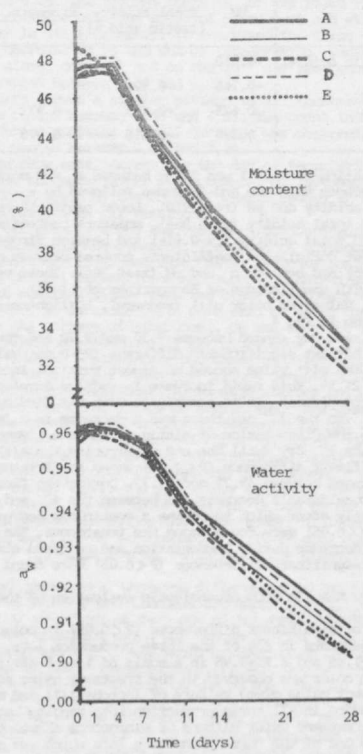

Fig.3. Changes in moisture content and water activity (based on three commercial lots)

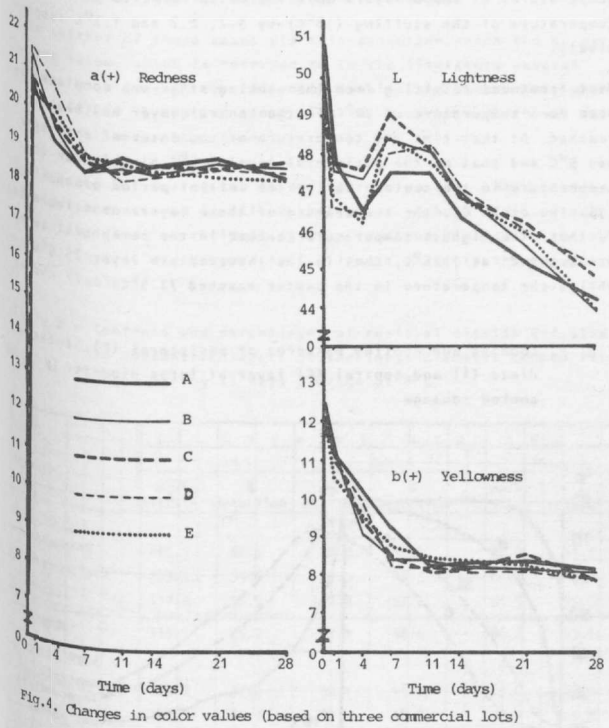

Fig.4. Changes in color values (based on three commercial lots)