Examination of the influence of curing brine ingredients on water activity (a_w) and multiplication of selected microorganisms

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Introduction

It is a known fact that for their biological functions, microorganisms use nutritive matters dissolved in water. According to Scott, however, microorganisms can use only a certain amount of the present water for which he introduced the term "water activity" (a_w). Numerous papers point at the significance of water activity in meat technology and in theoretical microbiology: Christian; Scott; Beers; Bem et al.; Christian and Waltho; Labuza; Riemann; Brownlie; Ohye et al.; Leistner et al.; Strong et al.; Tomčova et al.; Rödel et al. Besides other things, the results of these examinations establish optimum and minimum a_w- values necessary for the growth and miltiplication of different genera of bacteria, yeasts and moulds.

The way in which ingredients used in curing brines (common salt, phosphates, nitrites, nitrates and glycose) influence the a_w-value and the ability of meat microflora to multiply seems to be an interesting problem. Therefore the aim of this work was to examine the influence of adding these ingredients to nutritive bouillon on the a_w-value and on multiplication of selected microorganisms.

Materials and Methods

1) Microorganisms and media.- The authors used the following strains: Streptococcus faecalis, Staphylococcus aureus, Escherichia coli and Bacillus licheniformis (obtained from the collection of Bundesanstalt für Fleischforschung - Kulmbach).

Nutritive bouillon and agar were used as basic media whereas media with added ingredients were separately prepared. The selected ingredients were added to nutritive bouillon (having NaCl content = 0.5% and a_w = 0.9969) in such quantities as to obtain the following concentrations: NaCl - 0.5%, 2%, 5% and 10%; $NaNO_3$ - 0.05%, 0.1%, 0.5%, 0.73% and 1.0%; $NaNO_3$ + $NaNO_2$ - 0.05% + 0.005%; 0.1% + 0.01%; 0.5% + 0.05% and 1.0% + 0.1%; polyphosphate (Tari P_{22}) - 0.3% and 0.5%; and glycose - 0.5%, 1.0%, 2.0% and 3.0%.

In addition, "control bouillon" was prepared so as to have the same a_w (0.9969) as nutritive bouillon whereby such a_w-value was achieved by adding the following quantities of individual ingredients: 0.73% - $NaNO_3$; 1.2 + 0.12% - $NaNO_3$ + $NaNO_2$; 0.82% - Tari P_{22}; and 3.42% - glycose. The prepared media, in the quantity of 200 ml each, were poured into Erlenmeyer flasks and sterilized at 120°C for 30 minutes. In all media, water activity was determined with an instrument produced by the Luft company (Stuttgart), registering the a_w-value within the range from 0.4 to 1, at temperatures between 0 and 40°C, and the obtained results were checked by a mathematical method defined by Mirna (11).

2. Examination of multiplication.- All the selected microorganisms were examined simultaneously and under the same conditions. Media for the examination of the influence of added ingredients on the a_w-value and on multiplication of selected microorganisms were cultivated with 0.1 ml of the primary dilution of the known initial concentration. After 24-hour incubation in media of a different a_w-value, cultures of selected microorganisms were homogenized and afterwards 1 ml amounts of each medium were cultivated in triplicate on nutritive agar. The bacteria count was read after incubation at 37°C for 24 and 48 hours. Mean value of three cultivated Petri dishes was taken as total bacteria count for each of the examined microorganisms.

Results and Discussion

The influence of definite concentrations of curing brine ingredients on water activity and on multiplication of selected microorganisms is presented in Figures 1 through 5. The results of the influence of definite concentrations of sodium chloride on water activity and on multiplication of selected microorganisms show that concentrations of 2, 5 and 10%, namely water activity of bouillon from 0.9878 to 0.9420, effect the multiplication of Str. faecalis, Staph. aureus, E.coli and B.licheniformis. It can be said that at the concentration of 10% NaCl and an a_w of 0.9420, Str.faecalis, Staph. aureus and E.coli do not multiply after 24-hour incubation at 37°C; already at the concentration of 5% NaCl, namely an a_w of 0.97011, E.coli does not show growth. The most resistant microorganism is B.licheniformis which grew at all the examined concentrations but considerably less at concentrations higher than 5% NaCl. The results obtained for the concentration of 10% NaCl and an a_w of 0.9420 agree with the literature data (Leistner, Rödel, Bem, Tomčov) showing that the minimum a_w value necessary for the multiplication of Str. faecalis is 0.94. The same conditions do not allow multiplication of Staph.aureus and result in the reduction of initial count, what differs to a certain degree from the opinion of Scott, Bem, Tomčov and Rödel. In their opinion, this microorganism is not multiplied at an a_w of 0.91. On the basis of such statement it could be assumed that in addition to water activity in the substrate, there exist also an other simultaneous effect caused by chlorine iones from sodium chloride as well as by the change of the osmotic state of bouillon. The assumption that NaCl does not influence the multiplication of microorganisms by the change of a_w only but due to other effects as well, is confirmed by the results obtained in the examination of E.coli. In the literature (Karan-Djurdjić, Leistner, Mossel) it is reported that multiplication of E.coli stops at a_w 0.95. In our conditions of examinations, however, at a_w 0.97 obtained by the addition of 5% NaCl, the growth and reproduction of this microorganism was not established after incubation at 37°C for 24 hours.

Sodium nitrate reduces the a_w-value of bouillon whereas it does not influence essentially the examined microorganisms even at the concentration of 1%. Relatively low concentrations of $NaNO_3$ (0.05% and 0.1%), which change the a_w-value of bouillon to a minimum extent, retard the multiplication of Str. faecalis; such activity on Staph. aureus was expressed only at the concentration of 1%, whereas E.coli behaved contrary to that and multiplied better at higher concentrations, in spite of the reduced water activity. The behaviour of B.licheniformis was equal at all the examined concentrations of $NaNO_3$ regardless of different a_w-values of bouillon.

That is probably the reason why Hadži-Beganović believes that the activity of $NaNO_3$ on the reproduction of meat microflora is not of any particular interest.

Influence of the combinations of different concentrations of sodium nitrite and sodium nitrate, namely of the a_w-value of bouillon, on the multiplication of selected microorganisms is different. Inhibitory effect of these combinations appeared only at the relation of 0.1% $NaNO_2$ + 1% $NaNO_3$, when the a_w-value of bouillon is 0.9922. E.coli is resistant to the least extent, whereas Staph.aureus, Str.faecalis and B.licheniformis are less sensitive. Multiplication of the examined microorganisms was not observed in the control bouillon containing 0.12% $NaNO_2$ and 1.20% $NaNO_3$ and having the same a_w-value as nutritive boullon (0.9969). These results speak in favour of the opinion that the activity of the combinations of nitrate and nitrite is due to a specific phenomenon of these salts and not the result of the water activity complex. Such opinion is advocated by Lange who ascribes the inhibitory activity to the undissociated form of $NaNO_2$. The obtained results are of greater interest from theoretical standpoint of clarification of water activity effect on the ability of multiplication of microorganisms examined in this work than from the standpoint of practice, because efficient concentrations are 10, namely 100 times higher than the quantities used in meat curing brines. Tari P_{22}, in concentrations from 0.3 to 0.5%, did not influence more essentially the change of the a_w-value of bouillon, and its inhibitory activity was slight in all the examined microorganisms. The polyphosphate concentration of 0.5%, at the bouillon a_w-value of 0.9963, retards to some extent the growth of E.coli. Its concentration of 0.82% (control bouillon) retards very efficiently the growth of Str. faecalis, E.coli and B.licheniformis. There are many reasons for the statement that the activity of that quantity of polyphosphate on the reduction of the growth of the examined microorganisms is the result of a specific activity of certain fractions of this complex chemical composition, because the a_w-value is here 0.9969, namely the same as in nutritive bouillon. Total bacteria count of all the examined microorganisms is 100, namely 1000, and even 10000 times lower than in the corresponding nutritive bouillon after 24-hour incubation at 37°C.

The examined concentrations of glycose did not reduce more essentially the a_w-value of bouillon and the majority of the examined microorganisms grew well in its presence although they behaved differently. Str. faecalis bears well higher concentrations (2-3%) and E.coli shows better growth at 1% although even the quantity of 3.42% (control bouillon) does not influence more considerably its reproduction. All the examined concentrations of glycose reduce almost identically the ability of Staph. aureus to multiply. B.licheniformis bears better the minimum quantity of 0.5% as well as the quantity higher than 3%, in relation to concentrations from 1 to 2%. The literature data are to a certain degree contradictory to these results. Bem et al. explain the inhibitory activity of glycose by the change of the substrate a_w-value. In the opinion of Lange, the activity of glycose is dependent on the examined concentration and the microorganism kind. Some microorganisms are protected by its activity and enabled to multiply better whereas the others are inhibited.

The comparative review (Figure 6) of the growth of selected microorganisms in control bouillon, the same a_w-value of which was achieved by the addition of different quantities of the examined ingredients, shows significant differences in reproduction.

These results show that the a_w-value of 0.9969 is not necessarily favourable for the growth of microorganisms if curing brine salts, namely polyphosphates or glycose, are used instead of usually applied ingredients.

References

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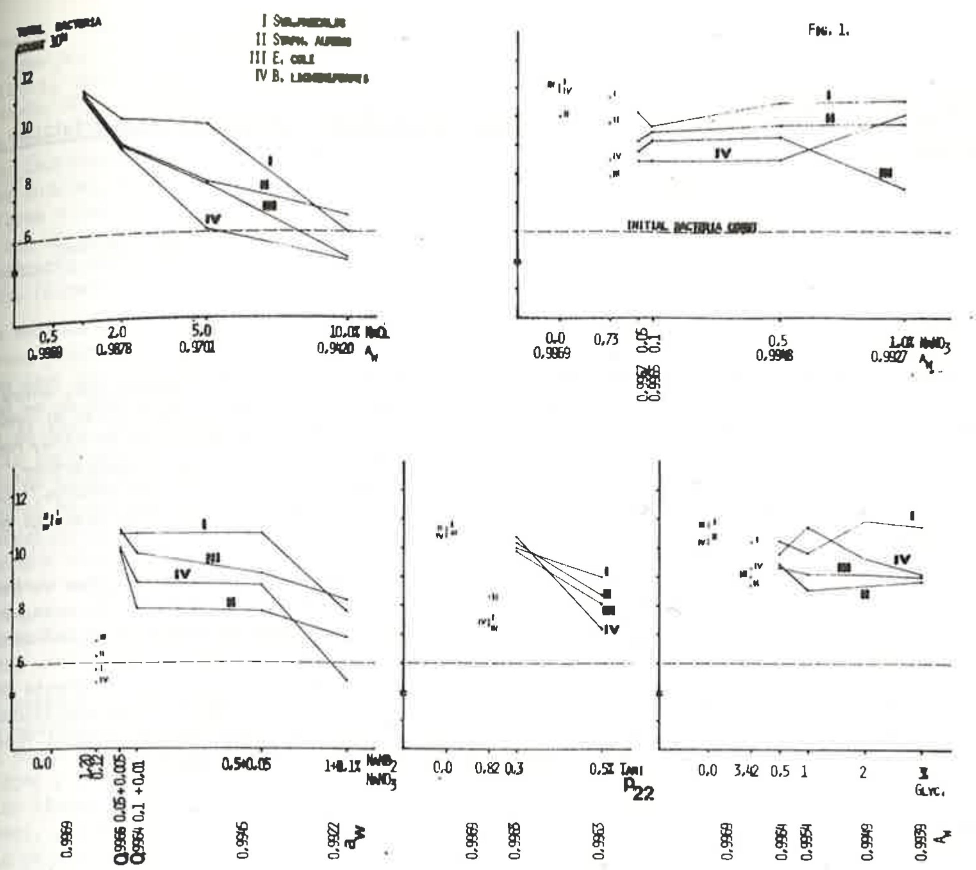
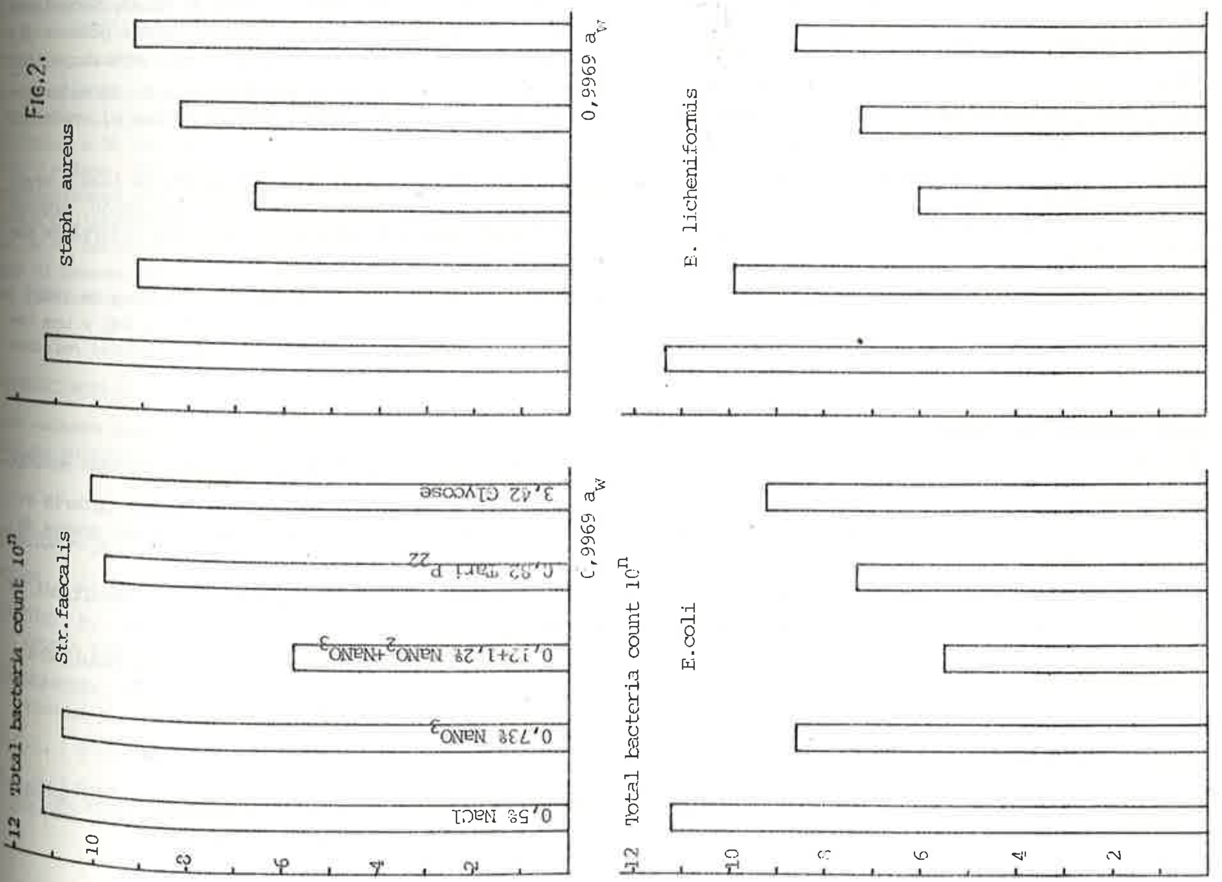
Fig.1. Influence of common salt sodium nitrate, concentrations of $NaNO_2$ and $NaNO_3$, Tari P_{22} and glucose on a_w and on multiplication of selected microorganisms.

Fig.2. Multiplication of selected microorganisms in bouillons having equal a_w-value adjusted by the addition of different quantities of the examined ingredients.


TOTAL BACTERIA COUNT 10ⁿ
I Str. faecalis
II Staph. aureus
III E. coli
IV B. licheniformis
INITIAL BACTERIA COUNT
NaCl
NaNO3
NaNO2
Glyc.
P22
aw
Fig. 1.


Staph. aureus
B. licheniformis
Str. faecalis
E. coli
Total bacteria count 10ⁿ
0,5% NaCl
0,73% NaNO3
0,12+1,2% NaNO2+NaNO3
0,82 Tari P22
3,42 Glycose
0,9969 aw
Fig. 2.