

Carlo Cantoni, Maria Rita Molnar, Pietro Renon e Giovanni  
Giolitti.

Behaviour of nucleotides, nucleosides and  
bases in dry sausages during ripening.

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Insituto di Ispezione degli Alimenti  
University of Milan  
Via Celoria, 10 - Milano - (Italy)

In recent years many papers have been published on the identification of meat and vegetable flavours and the importance of nucleotides as flavouring substances has been pointed out. The results of these investigations, performed mainly in the USA, Japan and the United Kingdom, have been recently reviewed by Kuninaka, Kibi and Sakaguchi (1964) Shimazono (1964) Craveri (1966) Tarr (1966) Kuninaka (1966).

On the basis of the investigations of a number of authors an increasing interest in food technology was given to flavour enhancers and especially to ribonucleotides, among them only 5' ribonucleotides possess characteristic taste, that is, 5' inosinic acid, 5' guanylic acid and 5' xanthilic acid. The 5' nucleotides are now produced by enzymatic degradation of ribonucleic acid or by chemical phosphorylation of inosine produced fermentatively. As regard meat products, the nucleotides present are mainly derived from ATP and are formed in the tissues after the animals have been slaughtered (Webster, 1953; Bendal and Davey, 1957; Howard, Lee and Webster, 1960; Millo, 1964; Solovev, 1966).

In a series of investigations carried out to detect the microbiological and chemical changes which occur during ripening of dry sausages we have examined the qualitative and quantitative behaviour of nucleotides, nucleosides and bases as there was a lack of information on dry sausages only fresh meats having been investigated.

#### Methods

For these investigations a uniform batch of sausages "Milano" type, expressly manufactured was used. They were made with pork meat 70%, pork fat 30%, sodium chloride 3%, sodium nitrate, pepper and spices, in natural horse casing.

The sausages had an average weight of 500 g, and were ripened under controlled conditions of temperature and humidity according to the process used at the factory.

Sausages were taken to be examined after 1, 3, 16, 29, 38, 48, 64 days of ripening and samples of 100 g. were used for these investigations for which the methods of Jones and Murray (1960, 1962, 1964) were used.

#### Results

In table 1 the amounts of the compounds which were isolated during ripening at the selected times are reported.

Among nucleotides only IMP was isolated. It was present only in the first period of ripening: 70 mg per 100 gr (fresh weight) on the first day, 22 mg per 100 gr (fresh weight) on the third day and only in traces after 16 days.

The amount of inosine, which was 84 micromoles per 100 g (fresh weight) at the beginning of ripening decreases constantly and inosine is no longer detectable after 29 days of ripening.

On the contrary the amount of hypoxanthine increases during ripening from 126 micromoles per 100 g. (fresh weight) on the first day of ripening to 545 micromoles per 100 gr (fresh weight) at the end of the ripening period. This increase does not depend on the loss of water as it may be shown from the figures calculated on the basis of the dry weight.

## Discussion

The changes concerning nucleotides, nucleosides and bases which occur in sausages during ripening cannot be compared with the data of other authors as they have examined meats of different animals just after slaughtering. In sausages we were unable to detect the presence of nucleotides as AMP, ADP, ATP, CMP, XMP with the methods employed. The only nucleotide present is IMP which disappears during the first period of ripening.

In our opinion the difference between our results and those of other authors may depend on the different age of meats: the sausages we have examined were made with pork meat which was kept for several days at 0°C before being manufactured and were examined during a long period of ripening (64 days) while the other investigators had used fresh meat.

The results of Millo (1964) seem to confirm our hypothesis: this author, studying the behaviour of nucleotides of the "longissimus dorsi" of pork at different times after slaughtering has noticed a strong reduction of ATP, ADP and AMP content of muscle after 30 hours and an increase in IMP which becomes the major component. Similar results were obtained by Jones and Murray (1962, 1964) in experiments performed with fish meat. Our investigations show that IMP decreases gradually while hypoxanthine increases during ripening (table 1). Inosine, which is present in the early stages of ripening has behaviour similar to that of IMP, it cannot be detected after 29 days of ripening.

The increase of hypoxanthine may be reasonably explained as the result of the dephosphorilation of IMP to inosine and of the further breakdown of the total inosine (either derived from IMP or naturally present) by the nucleosidases of the tissues and perhaps of the microorganisms which are present in a large amount in sausages.

On the basis of our results it seems that during ripening the cycle of the breakdown of nucleotides is accomplished and consequently dry sausages are devoid of this type of flavour enhancer; furthermore there is not yet any evidence that hypoxanthine or other bases could have any importance from this point of view.

We believe that flavouring substances in dry sausages mainly originate from products which have a structure other than nucleotides.

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## T A B L E 1

Behaviour of IMP, inosine and hypoxanthine during ripening of dry sausages

Time of Ripening (days)	Humidity (%)	IMP mg per cent (fresh weight)	Inosine micromoles per 100 g. (fresh weight)	Hypoxanthine micromoles per 100 g. (fresh weight)	Inosine + Hypoxanthine micromoles per 100 g. (fresh weight)	Inosine + Hypoxanthine micromoles per 100 g. (dry weight)
1	48	70	84	126	210	405
3	41,2	22	72	218	290	495
16	28	tr	36	324	360	500
29	23,5	abs	15	403	418	545
38	20,5	"	abs	530	530	665
48	19,4	"	"	580	580	720
64	19,4	"	"	545	545	679

Tr = traces  
abs = absent

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#### RIASSUNTO

Sono state determinate le variazioni quantitative e qualitative di nucleotidi, nucleosidi e delle basi durante la maturazione degli insaccati. Si sono isolati ed identificati IMP, inosina ed ipoxantina. Al termine della maturazione si rinviene solo ipoxantina.

#### SUMMARY

Quantitative and qualitative variations of nucleotides, nucleosides and bases were determined during ripening of dry sausages. IMP, inosine and hypoxanthine were isolated and identified. When ripening is complete only hypoxanthine was found.

#### RESUMÉ

On a déterminé les variations quantitatives et qualitatives des nucléotides, nucléosides et des bases pendant la maturation des saucissons crus. On a isolé et identifié: inosine monophosphate, inosine, hypoxantine. Cependant à la fin de la maturation on trouve seulement l'hypoxantine.

#### ZUSAMMENFASSUNG

Es wurden qualitativen und quantitativen Schwankungen der Nucleotiden, der Nucleosiden und der basen während der Wurstreifung untersucht. IMP, Inosin und Hypoxanthin konnten isoliert und identifiziert werden. Am Ende der Wurstreifung lässt sich nur hypoxantin finden.

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