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DETECTION OF MUTAGENICITY IN SOME CURED MEAT PRODUCTS USING AMES TEST

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Kariman M. Hassan[1,*], Mahmmod[1] R.M., Khallaf[1] M.F. and Lamyaa M. Sayed[2]

1- Food Sci. Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shobra 11241, Cairo, Egypt

2- Genetics Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shobra 11241, Cairo, Egypt

*Corresponding author: karimanomar42@gmail.com

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ABSTRACT

The use of food additives must be under control specially with the improvement of many diseases such as cancer disease which became the most threaten disease all over the world, although there had become more health aware and medical culture, many unhealthy food products are being consumed increasely, so it became very important to study some food product's mutagenicity. Detecting mutagenicity with short term assay with high percentage sensitivity are specifications available at Ames test with the mutated *Salmonella typhimiurium* strains and the reverse growth of the mutated bacteria was an indicator to the sample tested mutagenicity .The aim of this study is to evaluate the effect of adding sodium nitrite salt with various levels on mutagenicity in two of processed meat products (pastirma and luncheon) using Ames test. The results gave in the tested samples sign of mutagenicity at low concentrations and high reverse growth at higher concentrations , sodium nitrite extract gave highest mutagenicity at 10% (1.5 ml dose) concentration , pastirma extract gave highest mutagenicity at 10% concentration (2 ml dose) and luncheon extract gave highest mutagenicity at 100% concentration.

Key words: Nitrite, Pastirma, Luncheon, Mutagenicity, *Salmonella typhimurium*, Ames test, Cured, Meat products.

INTRODUCTION

Chemical additives and various spices are used in curing to improve the meat products. **(Asku et al 2016)**. Therefore, sodium nitrite used in cured meat products prevents anaerobic microorganisms such as *Clostridium botulinum*, delays the development of oxidative rancidity, improves meat flavor and stabilizes the colour of red meat **(Zahran and Kassem, 2011).**

Pastirma that considered as a traditional dry-cured, non-fermented raw meat product was considered intermediate moisture foods. Its name 'pastirma' from the Turkish verb 'Bastirma'

(**Mahmoud et al 2016)**. So**,** salting and or curing is the most important method affecting the quality of pastirma with their additives **(Asku et al 2016)**. Luncheon meat is one of the most acceptable food products and an important industrial meat product, it is cured by sodium nitrite, the risk of nitrites in luncheon meat resulting from transformation to nitrosamines which have a carcinogenic effect (**Kdous et al 2016)** .

Processed (nitrite-preserved) red meat additionally contains high concentrations of performed mutagenic nitroso compounds (NOC). Some added cereals, cured with salt and nitrite and heat processed. The formation of N-nitrose compounds from sodium nitrite during meat curing and the endogenous formation being caused as a result of high consumption of meat particulary processed meat is associated with increased prostate cancer risk (**John et al 2011).**

Colorectal cancer (CRC) is correlated with processed meat intake (including burger, ham, bacon, salami, and pastirma) in all reports. The increase of meat consumption leads to increase of CRC (**Santarelli et al 2008)**. Over intake red meats and cured meats, are very dangerous which can cause colorectal cancer. Ames test (Salmonella test) is a

cheap, short, high sensitivity with rodent carcinogenicity studies used to detect substances can cause genetic change **(Zou, 2014)**.

Ames/Salmonella/microsome mutagenicity test system investigates chemical food additives through reverse mutation by using the most sensitive *Salmonella* tester strains TA98 and TA100. Positive results were represented as an increase in the numbers of revertant colonies (**Hojati and Dehghanianb, 2014).**

MATERIALS AND METHODS

MATERIALS

Salmonella Enterica Ss. Enterica (Ex Kauffmann And Edwards) (Le Minor And Popoff Serover Typhimurium) was obtained from Cairo Mircen, Fac. of Agric., Ain Shams Univ., Cairo, Egypt.The expirement was done in Department of Genetics, Fac. of Agric., Ain shams Univ.

Meat products: Pastirma and luncheon meat products were purchased from local market at Cairo, Egypt. Samples of pastirma and luncheon were prepared in 3 concentrations (0.1, 10 and 100%) then the incubated strain exposed to different dosages of each concentration (1, 1.5 and 2 ml) .The bacterial growth measured by spectrophotometer on 600 nm (Hautefort *et al* 2003) ,

ANALYTICAL METHODS

Proximate composition of meat products (moisture, protein, ash, fibers and) was determined according to A.O.A.C. (2007), while fat content was determined as given by **(Bligh and Dyer , 1959)**. Sodium nitrite (NaNO2) was determined according to EPA 300.0 method at Agriculture Research Centre (ARC), Giza, Governorate, Egypt.

RESULTS AND DISCUSSION

1- Chemical composition of pastirma and luncheon

Data given in **Table (1)** showed approximate chemical composition of investigated meat products as well as sodium nitrite in meat products.

Moisture content was approximately the same, it was 54.6 and 58.6% for pastirma and luncheon, respectively. It is of interest to notice that protein content was higher in pastirma (72.5%) rather than that of luncheon product (9.8%).

It was higher in pastirma with 7.5 fold, rather than luncheon product. This is because pastirma was made of meat cut without any non meat ingredients like luncheon product which made from meat and non meat ingredients.

A contradicted trend was noticed in case of fat content ; i.e fat content was higher in luncheon rather than pastirma with 8.7 fold. This is because the addition of high percent of fat in luncheon recipe. Regarding to ash content it was higher in pastirma rather than luncheon by 2.13 fold. This is owing to higher percent of sodium nitrite that used for making pastirma (0.525%) rather than that its corresponding percent in luncheon 1.75% (Table1).Fiber content was higher in luncheon product with about 4 fold this is because various ingredients that added for making luncheon such as soybean , the results are in agreement with **(Çakıcı et al 2014).**

Table 1. Proximate chemical composition and sodium nitrites content of investigated meat products

Parameter	Meat product	
	Pastirma	Luncheon
Moisture	54.6%	58.6%
Protein	72.5%	9.8%
Fat	3.10%	27.17%
Ash	15.6%	7.3%
Fibers	1.11%	4.57%
Sodium nitrite	0.525%	1.75%

2- Mutagenicity effect of sodium nitrite

According to the results in **Table (2)**, the bacterial growth of control was (0.548) and it gave (0.349) in negative control.

- **0.1% salt concentration (100 ppm)**

The bacterial growth in 0.1% concentration gave sign of mutagenicity in (1ml) dose and increment of bacterial growth slightly higher than negative control,it gave (0.350) although it gave (0.349) in negative control in 1.5 dose the increment of bacterial measurement was noticeable, it gave (0.472) higher than the negative control and 1 ml dose , by increasing the dose of sodium nitrite (0.1 %) to 2 ml the bacterial measurement growth increased to (0.484) the increment of the bacterial reverse growth by adding 0.1% sodium nitrite salt and the increment of growth by increasing the salt dose indicates the mutagenicity of the sodium nitrite in 0.1% concentration.

- **10% salt concentration**

According to the following results in table 2 the bacterial growth of 1ml dose (10% concentration) increased to (0.632) higher than the 0.1% salt, the bacterial reverese growth gave the highest incensement in 1.5ml dose it gave (0.647), the effect of the 10% sodium nitrite is obviously mutagenic and more dangerous the bacterial reverse growth began to decrease to (0.585) by increasing the dose to 2 ml.

- **100% concentration**

The increment of salt concentration to 100% caused decrement of bacterial growth, the decrement reach to (0.325) by increasing the salt concentration dose to 2 ml, that because of the bacterial cell intolerance of the salt high osmotic pressure so cells died.

Table 2. Absorbance measurements of bacterial growth (reverse mutated by sodium nitrite)

Salt concentration	Control	Negative control	Sodium nitrite Salt Dose*		
			1 ml (d1)	1.5ml (d2)	2 ml (d3)
0.1% (c1)	0.548±.006[b]	0.349±.003[e]	0.350±.003[e]	0.472±.005[c]	.0.484±.002[c]
10% (c2)			0.632±.008[a]	0.647±.007[a]	0.585±.004[b]
100% (c3)			0.337±.02[e]	0.412±.03[d]	0.325±.02[e]

*all flasks with fixed volume (25ml) contain 1 ml of strain and different dosage sample.
Means followed by different small letters in the same row (effect of treatments) are significantly by Dunken's multiple tests ($p < 0.05$)

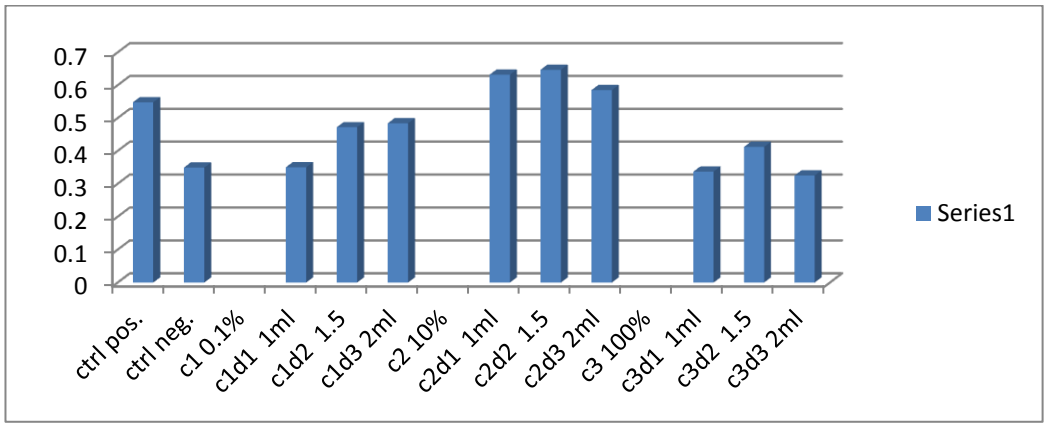

Fig. 1. Absorbance measurements chart of bacterial growth (reverse mutated by sodium nitrite)

3- Mutagenicity effect of pastirma sample extract

According to the results in **Table (3)**, the bacterial growth of control was (0.760) and it gave (0.513) in negative control.

- **0.1% pastirma extract concentration**

The bacterial growth in 0.1% concentration was (0.528) in (1ml) dose, it is higher than the negative control, it gave indicator to the mutagenicity of the pastirma sample in spite of the sample low concentration (lower than the authorized percentage). In 1.5 dose the increment of bacterial measurement was noticeable, it gave 0.516 higher than the negative control and 1 ml dose, by increasing the dose of the sample concentration dose (0.1 %) to 2 ml the bacterial measurement growth increased to (0.569) the increment of the bacterial reverse growth by adding 0.1% pastirma extract and the increment of growth by increasing the extract dose indicates the mutagenicity of the pastirma in 0.1% concentration.

- **10% pastirma extract concentration**

According to the following results in **Table (3)** the bacterial growth of 1ml dose (10% concentration) increased to (0.705) higher than the 0.1% salt, the bacterial reverse growth gave higher increment in 1.5ml dose it gave (0.708), the effect of the 10% sample extract 2 ml dose is obviously clear in 2 ml dose sample dose, that indicates the high mutagenic effect of the sample in 10% concentration.

- **100% pastirma extract concentration**

The increment of sample extract concentration to 100% caused decrement of bacterial growth, the decrement gave different measurements in the 3 doses and the effect of the sample increment was lower than the 0.1% and 10% concentration, that because of the bacterial cell intolerance of the salt high osmotic pressure so cells died.

Table 3. Absorbance measurements of bacterial growth (reverse mutated by pastirma extract)

Sample concentration	Control	Negative control	Pastirma Sample extract Dose *		
			1 ml (d1)	1.5ml (d2)	2 ml (d3)
0.1% (c1)	.760±.002^{a}	.513±.002^{f}	0.528±.003^{d}	0.516±.003^{f}	0.569±.0006^{c}
10% (c2)			0.705±.002^{b}	0.708±0.008^{b}	0.749±0.019^{a}
100% (c3)			0.508±0.002^{f}	0.531±.005^{d}	0.569±.0006^{c}

***all flasks with fixed volume (25ml) contain 1 ml of strain and different dosage sample.

Means followed by different small letters in the same row (effect of treatments) are significantly by Dunken's multiple tests ($p < 0.05$)

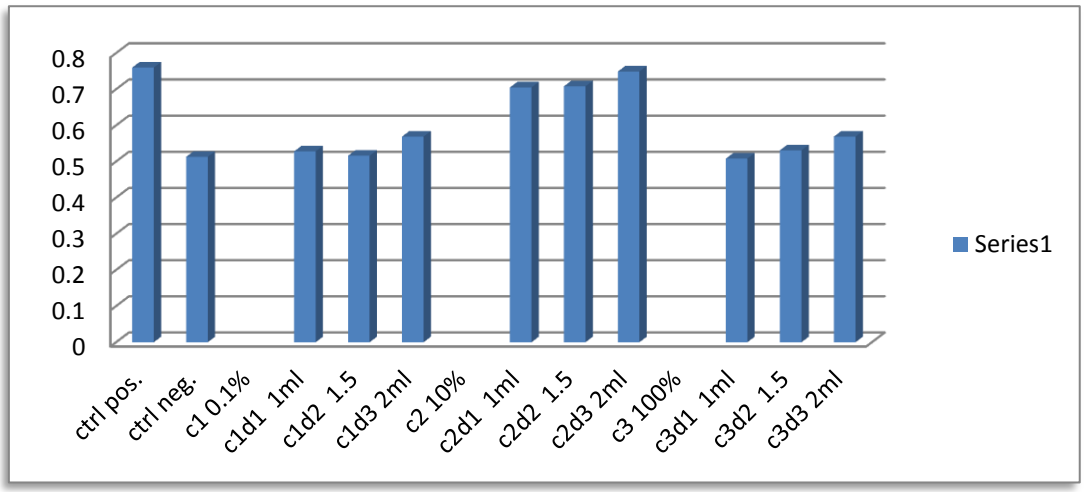

Fig. 2. Absorbance measurements chart of bacterial growth (reverse mutated by pastirma extract)

4- Mutagenicity effect of luncheon sample extract

According to the results in **Table (4)**, the bacterial growth of control was (0.695) and it gave (0.247) in negative control.

- **0.1% luncheon extract concentration**

The bacterial growth in 0.1% concentration gave sign of mutagenicity in (1ml) dose and increment of bacterial growth slightly higher than negative control in 1.5 dose the increment of bacterial measurement was noticeable, it gave 0.340 higher than the negative control and 1 ml dose, by in-

creasing the dose of the sample concentration dose (0.1 %) to 2 ml the bacterial measurement growth increased to (0.355), that indicates the mutagenicity of the sample extract in spite of the low concentration.

- **10% luncheon extract concentration**

According to the following results in **Table (4)** the bacterial growth of 1ml dose (10% concentration) increased to 0.399 higher than the 0.1% salt, the bacterial reverse growth gave higher increment in 1.5ml dose it gave 0.464, the effect of the 10% sample extract 2 ml dose is obviously clear in 2 ml sample dose it gave 0.480 higher than the previous concentration, that indicates the mutagenic effect of the sample in 10% concentration.

- **100% luncheon extract concentration**

The increment of the bacterial growth continued the increment by increasing the sample extract to 100% concentration, it gave the highest reverse growth in 2 ml dose.

The previous results are in agreement with (**Zou 2014)**.

Table 4. Absorbance measurements of bacterial growth (reverse mutated by luncheon extract)

Sample concentration	Control	Negative control	luncheon Sample Dose*		
			1 ml (d1)	1.5ml (d2)	2 ml (d3)
0.1% (c1)	0.695±.004[a]	0.247±.08[g]	0.249±.007[g]	0.340±.007[f]	0.355±.009[f]
10% (c2)			0.399±.002[f]	0.464±.02[d]	0.480±.007[d]
100% (c3)			0.538±.03[b]	0.513±.008[d]	0.543±.008[b]

***all flasks with fixed volume (25ml) contain 1 ml of strain and different dosage sample.

Means followed by different small letters in the same row (effect of treatments) are significantly by Dunken's multiple tests ($p < 0.05$)

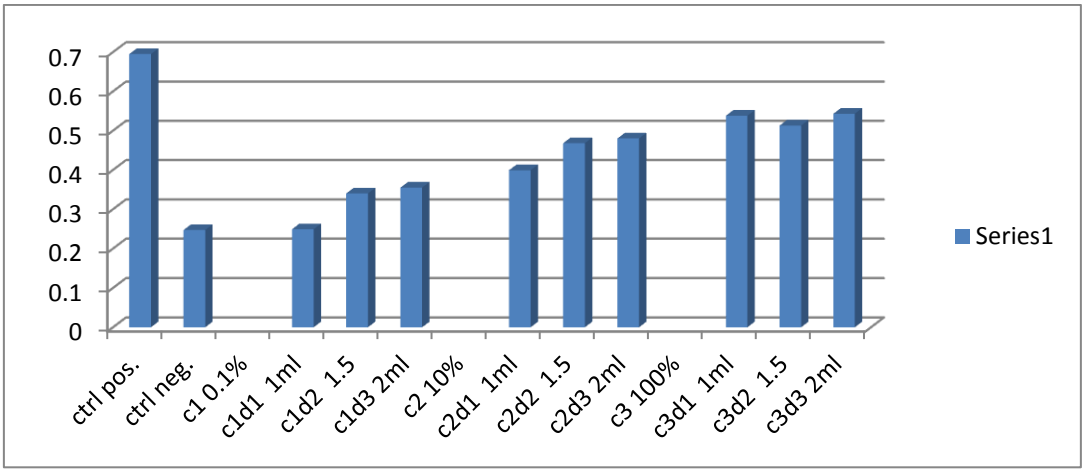

Fig. 3. Absorbance measurements chart of bacterial growth (reverse mutated by luncheon extract)

CONCLUSION

-Sodium nitrite is a mutagenic salt, using it as a food additive is dangerous even in low concentrations.
-Consuming of luncheon and pastirma must be limited as possible to avoid the dangerous of cancer.
-Curing meats with sodium nitrite is very dangerous and there must be alternatives to this salt .

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الكشف عن الطفرات الحادثة في بعض منتجات اللحوم المعالجة باستخدام Ames test [177]

كريمان محمد حسن[1،*] – رمضان محمد محمود[1] – محمد فرج خلاف[1] – لمياء مصطفي كمال سيد[2]

1- قسم علوم الأغذية – كلية الزراعة – جامعة عين شمس – ص ب 68 – حدائق شبرا 11241- القاهرة – مصر

2- قسم الوراثة – كلية الزراعة – جامعة عين شمس – ص ب 68 – حدائق شبرا 11241- القاهرة – مصر

*Corresponding author: karimanomar42@gmail.com

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الموجـــــــز

مع تزايد انتشار العديد من الامراض مثل السرطان والذي أصبح اكثر مرض يهدد البشرية علي مستوي العالم، كان لابد من التحكم في استخدام الاضافات الغذائية وعلي الرغم من تزايد التوعية الصحية والثقافة الطبية أصبح استهلاك العديد من الأطعمة غير الصحية في تزايد، لذا أصبح من الأهمية دراسة حدوث الطفرة في المنتجات الغذائية. الكشف عن الطفرات باستخدام اختبار قصير المدة الزمنية وعالي الحساسية هي مواصفات متوفرة في اختبار السالمونيلا باستخدام سلالات بكتريا مطفرة ويعتبر النمو المنعكس للبكتريا المطفرة مؤشر لوجود طفرة بالعينة المختبرة. لذا تهدف هذه الدراسة الي تقييم تأثير اضافة ملح نيتريت الصوديوم بمستويات مختلفة علي احداث طفرة في نوعين من اللحوم المعالجة (البسطرمة واللانشون).

وأعطت النتائج مؤشرا لوجود طفرة مع التركيزات المنخفضة وارتداد عالي للنمو مع التركيزات العالية، اعطي مستخلص نيتريت الصوديوم اعلي طفرة في تركيز 10% (جرعة 1.5 مل) ،مستخلص البسطرمة أعطت اعلي طفرة عند تركيز 10% (جرعة 2 مل) ومستخلص اللانشون أعطي اعلي طفرة عند تركيز 100%.

الكلمات الدالة: نيتريت، بسطرمة، لانشون، طفرة، اختبارالسالمونيلا، منتجات اللحوم المعالجة

تحكيم: ا.د سمير يوسف عبده السناط
ا.د يسـري احمد عبد الدايـم