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Abstract

Processed meat products are presumptive sources of mutagens and genotoxins for consumers. Several epidemiological studies have reported that these products' high intakes have a positive link with cancer incidence. In Algeria, industrially processed meat products are widely consumed. However, there are no earlier studies involving their genotoxic activity. For this end, the current study aimed at evaluating the mutagenicity and the genotoxicity of some representative industrially processed meat products sold in popular supermarkets. All samples were extracted by established method, using both polar and non-polar solvents. The meat extracts mutagenicity was assessed by Ames test, using four strains of Salmonella typhimurium in the presence and absence of metabolic activation, and subsequently by treat and wash assay for extracts showing biologically significant results. The genotoxicity was determined in TK6 human lymphoblastoid cells using the *in vitro* micronucleus assay in micromethod. The results showed that all extracts analyzed induce no mutagenic activity. However, one of these extracts induced a biologically significant increase in the number of micronucleated cells. Our findings indicate the importance of the genetic damage detection for taking measures to suppress or reduce the exposure to harmful contaminants and encourage further research investigating genotoxic effects of industrially processed meat worldwide.

1 Introduction

Meat is a valuable food and is an important source of protein of high biological value, essential amino acids, vitamins and iron (Domingo and Nadal, 2016). However, it is well-established that its transformation and preservation, by chemical and/or physical methods, have the potential to generate mutagenic and genotoxic agents (Ruan et al., 2014). It has become evident that these compounds have a high degree of correlation with carcinogenicity (McCann et al., 1975). Several epidemiological studies have also shown that there is a causal relationship between the consumption of meat, especially in processed form, and the appearance of several types of cancer notably colorectal, gastric, esophageal and bladder cancers (Cross et al., 2007; Deoula et al., 2019; Lippi et al., 2016). Indeed, the International Agency for Research on Cancer has been classified the processed meat as carcinogenic to humans (Group 1)(IARC, 2018).

There have been many reports regarding the mutagenic effects of cooking meat which is prepared by grilling, frying, boiling or broiling (Bjeldanes et al., 1983; Commoner et al., 1978; Nagao et al., 1977; Omoruyi et al., 2014; Pariza et al., 1979). However, very little attention has been focused on the mutagenic and genotoxic potential of industrially processed foods, particularly processed meat products. Krone and Iwaoka (Krone and Iwaoka, 1984) reported for the first time the mutagenic activity of some commercially canned meats like corned-beef, beef broth and roast beef. Study in Finland by Omoruyi and Pohjanvirta (Omoruyi and Pohjanvirta, 2014) showed also that industrially processed, cold-smoked beef, smoked chicken and grilled turkey induce mutagenic activity in Ames test with the TA 100 *Salmonella typhimurium* strain with and without metabolic activation. Therefore, these products appear to be a probable important source of mutagens. In Algeria, the low production and high prices of meats lead a large majority of consumers to move towards the

consumption of industrially processed meat products, which may constitute a risk for human health (Chikhi and Bencharif, 2016). The fact of the high consumption of these products available in various flavors and low cost when compared to raw meats, emphasizes the importance of investigating their mutagenic and genotoxic activity. Furthermore, food additives like coloring agents, flavoring agents and preservatives might be the potential cause of genotoxicity (Nepalia et al., 2018). On the other hand, the processing / cooking methods of meat such as smoking, roasting and grilling might also be responsible for genotoxic compounds formation. Among these genotoxic compounds, polycyclic aromatic hydrocarbons and heterocyclic aromatic amines which are produced at high and mild cooking temperatures, respectively (Sanz-Serrano et al., 2020).

Even though mutagen substances may appear during household cooking, the consumers can reduce their formation using lower temperature methods like steaming, boiling or microwave heating. While, this opportunity is not available to the consumers of industrially processed meat products (Krone and Iwaoka, 1987). Hence, if these final products exhibit any genotoxic activity, they can cause deleterious effects for consumers.

In this context, the present study aimed at evaluating the potential mutagenic and genotoxic effects of polar and non-polar extracts of some industrially processed meat products currently available in Algerian supermarkets using the Ames test on four *S. typhimurium* strains together with complementary test "treat and wash test" and the *in vitro* micronucleus test on the TK6 human lymphoblastoid cell line, respectively.

2 Materials and methods

2.1 Chemicals

Chemicals used in this study were purchased from the following suppliers: dimethyl sulfoxide (DMSO) from Acros Organics (Noisy le Grand, France); RPMI 1640 medium, horse serum,

non-essential amino acids and phosphate-buffered saline (PBS) from GIBCO Invitrogen SARL (Cergy-Pontoise, France); distilled water from Fresenius (Bad Homburg, Germany); HCl, L-glutamine, Na_2HPO_4, absolute ethanol and 2-nitrofluorene from Merck (Darmstadt, Germany); acetic acid, NaH_2PO_4 and $MgCl_2$ from Merck VWR (Fontenay-sous-Bois, France); oxoid nutrient broth N°2 and Agar from Oxoid (Basingstoke, United Kingdom); glucose-6-phosphate and NADP from Roche (Mannheim, Germany); sodium bicarbonate, L-histidine, biotin, Giemsa stain, sodium pyruvate, penicillin, streptomycin, pluronic acid, KCl, NaCl, amphotericin B, 3-[4,5-dimethylthiazol- 2-yl]-2,5-diphenyltetrazolium bromid (MTT), benzo[a]pyrene, cyclophosphamide, sodium azide, 9-amino-acridine, griseofulvin and mitomycin C from Sigma–Aldrich (Saint-Quentin Fallavier, France); 2-anthramine and 4-nitroquinoline-N-oxide (4-NQO) from Sigma–Aldrich GmbH (Stein-heim, Germany).

2.2 Metabolic activation system

After enzymatic induction with Arochlor 1254, rat liver S9 was prepared according to Ames *et al.* (Ames et al., 1975) and Maron and Ames (Maron and Ames, 1983).

In the bacterial mutation assay, one mL of S9-mix contained 0.1 mL of S9, 0.02 mL of $MgCl_2$ (0.4 M), 0.02 mL of KCl (1.65 M), 0.5 mL of phosphate buffer (0.2 M, PH 7.4), 0.04 mL of NADP (0.1 M), 0.005 mL of glucose-6-phosphate (1 M) and 0.315 mL of distilled water.

In the *in vitro* micronucleus assay, one mL of S9-mix contained 0.4 mL of S9, 0.2 mL of KCl (150 mM), 0.2 mL of NADP (25 mg/mL) and 0.2 mL of glucose-6-phosphate (180 mg/mL). Except S9, all co-factors were filtered through a 0.45µm sterilizing membrane before use. S9-mix was used under a volume of 5%, for a final S9 concentration of 2%.

2.3 Sampling

A total of eight industrially processed meat products (corned-beef made in Algeria, imported corned-beef, salami, K-chir, smoked roast chicken, smoked roast beef, chicken chawarma and beef jambon) were purchased from a popular supermarket in Algeria. All these products are directly consumed, without any cooking process. In this study, one batch per product was analyzed. The ingredients and packaging of each product are listed in the Table I. We carefully confirmed that all meat products were extracted before the expiry date revealed on the packages.

Table I. Packaging and ingredients of industrially processed meat products.

Meat products	Packaging	Ingredients
Corned-beef made in Algeria	Canned	Frozen ground beef, salt, nitrite (0.6% sodium nitrite), food additives: gelling agent (carrageenan), stabilizers (sodium Di, Tri and polyphosphate) 2000 mg/Kg.
Imported corned-beef	Canned	Beef, cereals, salt, sugar and preservative INS 250.
Salami	Vacuum-packed	Beef, water, corn starch, nitrite salt (sodium nitrite), spices, table salt, dextrose, food additives: stabilizer (sodium polyphosphate), preservatives (sodium acetate and sodium diacetate), coloring agent (Allura red AC), flavor enhancer (monosodium glutamate, disodium guanilate, disodium inosinate), acidifier (citric acid), antioxidant (sodium lactate, ascorbic acid), anti-caking agent (silicon dioxide).
K-chir	Vacuum-packed	Chicken meat, water, corn starch, green olive, vegetable oil, beet, nitrite salt (0.6% sodium nitrite), soy protein, flavor extract (Di and Tri phosphate, monosodium glutamate, dextrose, soy, spices, sodium erythorbate, citric acid, onions), food additives: stabilizer (sodium polyphosphate 2000 mg / Kg), antioxidants (sodium citrate), Color (Allura red AC 25 mg / Kg).
Smoked roast chicken	Vacuum-packed	Chicken escalope, mechanically separated chicken, potato starch, corn starch, mixed spices, nitrite salt, food additives: stabilizers (INS 451 i, INS 450 i), acidifiers (INS 325, INS 330).
Smoked roast beef	Vacuum-packed	Fresh beef, salt, water, spice blend, food additives: stabilizer (sodium diphosphate).
Chicken chawarma	Vacuum-packed	Chicken meat, water, corn starch, nitrite salt (sodium nitrite), spices, table salt, dextrose, food additives: stabilizer (sodium polyphosphate), preservative (sodium acetate and sodium diacetate),

		flavor enhancer (monosodium glutamate, disodium guanilate, disodium inosinate), acidifier (citric acid), antioxidant (sodium lactate, ascorbic acid), anti-caking agent (silicon dioxide).
Beef jambon	Vacuum-packed	Beef, vegetable protein, edible oil, potato starch, mix of spices, herbs, stabilizer E450 AC, food coloring E120, beef flavoring.

2.4 Sample preparation

Industrially processed meat samples were extracted in the non-polar phase by using n-hexane and in the polar phase by using methanol (Sharif et al., 2008; Takahashi et al., 1979). Each sample (about 200 g) was minced and then extracted with 800 mL of methanol or n-hexane. The mixture was left on stirring overnight at room temperature. Then it was separated by centrifugation at 3000 rpm for 15 min, followed by double filtration through Whatman No. 1 filter paper. This procedure was repeated twice with 400 mL of the same solvents. The filtrates were pooled and evaporated using a rotary evaporator. Hexane extracts were reconstituted in ethanol, while methanol extracts were reconstituted in DMSO for *in vitro* analyses.

2.5 Bacterial mutagenicity assays

The mutagenic activity of meat extracts was evaluated initially by the standard plate incorporation assay. Extracts showing a significant outcome in this assay were subjected to treat-and-wash assay to check to what degree a release of histidine, peptides and/or proteins from the sample contributed to the result.

2.5.1 Standard plate incorporation assay

The standard plate incorporation assay, with and without metabolic activation, was performed using the histidine-requiring *S. typhimurium* strains as described by Ames et al. (Ames et al., 1975), Maron and Ames (Maron and Ames, 1983) and OECD Guideline No.471 (OECD,

1997) except that 4 strains were used instead of 5 (TA1537, TA98, TA100 and TA102). The tester strains were grown in Oxoid nutrient broth No. 2 at 37 °C, with shaking (120 rpm), for 12-13 h to obtain a final concentration of 1-2.10^9 cells/mL. Without metabolic activation, a 0.1 mL aliquot of this fresh culture and 0.1 mL of the test substance were successively added to 2 mL of molten top agar containing 10 % of 0.5 mM biotin/histidine solution. This mixture was then agitated and poured on glucose minimal agar plates. The Plates were incubated for 48 h at 37 °C. The method with metabolic activation was the same except that immediately before spreading in the plates, 0.5 mL of the S9-mix was added in top agar, *i.e.* 2% of S9 in final concentration.

Five different doses of the meat extracts were tested: 50, 150, 500, 1500 and 5000 µg per plate for methanol extracts, i.e. up to the recommended dose and 10, 30, 100, 300 and 1000 µg per plate for n-hexane extracts. The top dose was limited by the solubility of the extracts in the solvent. DMSO or ethanol was used as negative controls for all strains. Positive controls were assayed concurrently [without S9-mix:9-amino-acridine (50 µg/plate) for TA1537, 2-nitrofluorene (2 µg/plate) for TA98, sodium azide (1 µg/plate) for TA100, mitomycin C (0.125 µg/plate) for TA102; with S9-mix: 2-anthramine (2 µg/plate) for TA1537, TA98 and TA100 and benzo[a]pyrene (2 µg/plate) for TA102]. Triplicate plates were used for the test samples and positive controls, while sextuplicate plates for negative controls. Histidine-revertant colonies were counted 48 h after treatment. A response is considered positive in the assay if a dose-response relationship is observed on three consecutive doses with, for the highest increase, an induction ratio greater than or equal to 2 (TA98, TA100 and TA102) or 3 (TA1537) (Mortelmans and Zeiger, 2000). In parallel, data were analyzed by means of Dunnett's method (Mahon et al., 1989) allowing the comparison of the values for each dose with the values for the corresponding negative control.

2.5.2 Treat-and-wash assay

The treat and wash assay was conducted according to the method described by Thompson et al. (Thompson et al., 2005). The following were successively added to a sterile 15 mL tube: 0.05 mL of bacterial culture, 0.5 mL of S9-mix or phosphate buffer and 0.05 mL of sample extract dilution. This mixture was incubated for 90 min at +37°C with stirring. Then, 15 mL of a wash solution of Oxoid No. 2 nutrient broth in phosphate buffered saline (1:7 v/v) were added and the washed bacteria were collected by centrifugation at 3000 rpm for 30 min. All but approximately 0.7 mL of the supernatant was removed and discarded, and the bacteria were resuspended in the residual supernatant prior to plating via top agar.

2.6 *In vitro* micronucleus assay in micromethod

The *in vitro* micronucleus assay in micromethod was carried out as previously detailed by Nesslany et al. (Nesslany and Marzin, 1999) except that TK6 human lymphoblastoid cells were used instead of L5178Y mouse lymphoma cells.

2.6.1 Cells

The TK6 human lymphoblastoid cell line was obtained from the American Type Culture Collection (Rockville, USA) and has been described previously (Honma, 2005; Liber and Thilly, 1982; Skopek et al., 1978). This cell line is derived from the spleen of a patient with hereditary spherocytic anaemia and has a number of properties that are advantageous for mutagenicity and genotoxicity studies: a stable genome, stable spontaneous mutation frequencies, a functional p53 protein, and the ability to grow in suspension culture. The average doubling time of the TK6 cells was 16–18 h.

2.6.2 Cell culture

The TK6 human lymphoblastoid cells were cultured in RPMI 1640 growth medium (RPMI 0), which was supplemented with 10 % (v/v) heat-inactivated horse serum, 2 mg/mL sodium bicarbonate, 10 mL/l non-essential amino acids, 200 U/mL penicillin, 50 µg/mL streptomycin, 2.5 µg/mL amphotericin B, 200 µg/mL l-glutamine, 200 µg/mL sodium pyruvate and 500 µg/mL pluronic acid (RPMI 10 medium). Cells were maintained in tissue-culture flasks at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h.

2.6.3 Cell treatment

Exponentially growing TK6 cells, at a density of 6.10^5 cells/mL, were treated in 96-well microplates with different doses of the sample extracts. Each treatment was conducted in duplicate and was coupled to cytotoxicity assessment. Three types of treatment were performed:

a) In the first treatment, the cells were treated in the presence of metabolic activation for 3 h followed by a recovery period of 24 h (+S9, 3 h/+24 h).

b) In the second, the cells were treated without metabolic activation for 3h followed by recovery period of 24 h (-S9, 3 h/+24 h).

c) In the last one, the cells were continuously treated without metabolic activation for 27 h and were harvested immediately (-S9, 27 h/+ 0 h).

At the end of each treatment or recovery period, the microplates were centrifuged for 6 min at 1000 rpm. The cells were washed (180 µl, RPMI 0 culture medium + 0.1 % pluronic acid), gently resuspended and centrifuged. The cells were then treated for 4 min with 150 µl of hypotonic solution (RPMI 0 diluted 1:1, v/v in distilled water + 0.1 % pluronic acid) and pre-fixed by adding 50 µl of cold Carnoy's fixative (absolute ethanol/acetic acid, 3:1, v/v). After centrifugation, the cells were fixed with 150 µl of Carnoy's fixative for at least 10 min at

room temperature, then 24 h at 4°C. Subsequently, the cells were re-suspended, dropped onto clean glass slides and dried at room temperature at least overnight. Finally, air- dried slides were stained for 10 min with 2 % Giemsa aqueous solution, rinsed with distilled water and dried at room temperature. Positive controls [without S9-mix: mitomycin C at 0.5 µg/mL (3 h/+24 h treatment), mitomycin C at 0.2 µg/mL and griseofulvin at 5 µg/mL (27 h/+ 0 h treatment); with S9-mix: cyclophosphamide at 5 µg/mL] were included in each corresponding treatment schedule. Micronuclei were counted in at least 2000 intact mononucleated cells for the three retained doses (1000 mononucleated cells per slide) at 500 × magnification. The identification of micronuclei was conducted according to previously described by Miller et al. (Miller et al., 1995). The statistical significance of differences between doses was determined using the CHI2-test. Differences were considered statistically significant ($p < 0.05$). A product is classified as genotoxic to TK6 cells if at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control, the increase is dose-related in at least one experimental condition when evaluated with an appropriate trend test and any of the results are outside the distribution of the historical negative control data.

2.6.4 Cytotoxicity assay

The Cytotoxicity was evaluated using a 3-[4,5-dimethylthiazol- 2-yl]-2,5-diphenyltetrazolium bromid colometric method (Borenfreund et al., 1988). Metabolically active mitochondrial dehydrogenases convert the tetrazolium salt MTT to insoluble purple colored formazan crystals. The extent of formazan formed is proportional to cell viability. After the treatment, cells were centrifuged, washed with RPMI 0 and the MTT solution (5 mg/mL in Dulbecco medium without phenol-red) was added to each well in a volume of 100 µl. Plates were then incubated for an additional 2 hours at 37°C in the dark. Thereafter, MTT solution was

discarded and 100 µl of HCl/isopropanol solution (1:11.5, v/v) was added in order to dissolve the formazan products. Culture plates were gently shaken for 15 min. Finally, the absorbance was read at 550 and 620 nm using a spectrophotometer microplate reader (Thermo Labsystems, USA). To exclude cytotoxicity as a confounding factor, the cell viability should be equal or more than 55 ± 5 %. At least six doses were retained for genotoxicity assay.

3 Results

3.1 Standard plate incorporation assay

The mutagenic activity of polar and non-polar extracts of industrially processed meat products, expressed as the number of revertant colonies per plate, is presented in Table II and Table III.

Table II. Mutagenic activity of methanol extracts of industrially processed meat products.

Methanol extracts	Doses µg/plate	Number of revertant colonies (Mean ± standard deviation (SD))							
		TA1537		TA98		TA100		TA102	
		- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
Corned-beef made in Algeria	0[a]	10.3 ± 2.3	14.5 ± 6.2	32.0 ± 3.2	35.0 ± 5.3	103.0 ± 10.7	85.5 ± 9.6	203.3 ± 48.7	226.3 ± 39.7
	50	8.7 ±3.8	9.7 ± 3.1	28.0 ± 6.6	33.3 ± 2.9	106.0 ± 10.8	76.3 ± 6.0	234.7 ± 11.4	399.3 ± 8.3*
	150	13.7 ± 3.1	13.3 ± 6.7	24.3 ± 6.5	39.0 ± 2.0	112.0 ± 17.5	90.0 ± 6.0	250.3 ± 24	420.0 ± 68.1*
	500	10.7 ± 2.5	10.0 ± 5.3	47.3 ± 15.9	46.7 ± 4.6	126.3 ± 15.5	94.3 ± 11.1	335.7 ± 22.9*	400.7 ± 98*
	1500	14.0 ± 4.4	14.7 ± 7.6	41.3 ± 3.8	58.3 ± 12.9*	175.7 ± 16.1*	137.0 ± 28.4	342.0 ± 44.5*	422.0 ± 39.9*
	5000	ov	ov	ov	ov	185.3 ± 30.1*	152.7 ± 55.1*	ov	ov
Positive control	(b)	484 ± 144.3*	236.7 ± 13.3*	274.7 ± 25.8*	1658.7 ± 247.1*	874.7 ± 208.7*	1461.3 ± 219.7*	1394.7 ± 552.1*	528.0 ± 77.1*
Imported corned-beef	0[a]	9.0 ± 2.4	6.7 ± 2.5	14.8 ± 2.1	22.7 ± 4.9	106.5 ± 6.8	89.8 ± 7.1	156.0 ± 16.6	167.8 ± 36.9
	50	10.7 ± 5.0	5.3 ± 2.1	13.7 ± 2.9	24.0 ± 4.6	112.7 ± 7.5	106.7 ± 7.4	162.7 ± 3.1	238.7 ± 68.2
	150	10.7 ± 3.1	5.0 ± 1.0	15.0 ± 1.7	21.0 ± 4.6	96.3 ± 1.5	103.0 ± 11.1	146.0 ± 14.0	219.3 ± 38.2
	500	6.3 ± 1.2	5.3 ± 2.9	13.0 ± 2.6	24.3 ± 2.1	94.0 ± 12.3	93.7 ± 9.6	134.7 ± 17.2	270.7 ± 45.0*
	1500	3.3 ± 0.6	4.0 ± 1.7	21.0 ± 4.6	27.0 ± 2.0	81.0 ± 8.7*	101.7 ± 11.4	164.0 ± 26.2	332.0 ± 2.0*
	5000	3.7 ± 1.5	9.0 ± 5.0	17.3 ± 5.5	46.0 ± 1.7*	86.0 ± 1.7	145.3 ± 3.1*	81.7 ± 5.8*	390.0 ± 22.7*
Positive control	(b)	290.7 ± 97.2*	326.7 ± 137.2*	482.7 ± 24.4*	1536.0 ± 84.7*	302.7 ± 54.5*	1509.3 ± 33.3*	960.0 ± 69.7*	725.3 ± 128.3*
Salami	0[a]	10.5 ± 2.4	13.5 ± 4.0	24.3 ± 6.1	23.8 ± 9.2	99.2 ± 13.1	91.0 ± 12.0	194.5 ± 25.6	223.0 ± 31.3
	50	11.7 ± 1.2	15.7 ± 3.2	22.7 ± 4.7	25.3 ± 1.5	97.7 ± 4.0	106.3 ± 9.6	230.0 ± 55.2	263.0 ± 19.5
	150	11.0 ± 5.3	16.7 ± 2.3	28.3 ± 3.1	29.0 ± 4.4	106.3 ± 10.8	107.7 ± 6.7	277.3 ± 9.0	415.3 ± 17.2*
	500	9.0 ± 2.6	13.7 ± 3.1	29.0 ± 3.6	25.3 ± 3.5	112.7 ± 4.9	103.7 ± 5.0	376.0 ± 45.7*	386.0 ± 25.0*
	1500	10.0 ± 5.3	12.3 ± 4.0	29.0 ± 3.6	33.0 ± 1.7	129.0 ± 7.2*	125.3 ± 13.8*	364.7 ± 44.4*	470.7 ± 68.9*
	5000	8.3 ± 2.5	13.7 ± 5.5	28.0 ± 6.1	34.3 ± 6.7	217.3 ± 16.9*	129.7 ± 8.0*	ov	ov
Positive control	(b)	316.7 ± 80.8*	204.7 ± 24.2*	271.3 ± 26.4*	2650.7 ± 547.8*	561.3 ± 74.3*	2800.0 ± 688.0*	874.7 ± 124.0*	869.3 ± 203.9*

	0[a]	10.5 ± 2.4	13.5 ± 4.0	24.3 ± 6.1	23.8 ± 9.2	99.2 ± 13.1	91.0 ± 12.0	194.5 ± 25.6	223.0 ± 31.3
K-chir	50	9.7 ± 4.0	17.0 ± 5.6	19.3 ± 4.9	31.0 ± 6.2	104.0 ± 15.1	96.0 ± 19.2	214.7 ± 18.6	285.3 ± 14.5
	150	8.7 ± 2.1	20.3 ± 5.5	17.7 ± 3.1	20.3 ± 6.4	92.7 ± 6.7	92.3 ± 11.2	271.3 ± 23.9	273.3 ± 44.1
	500	8.0 ± 1.7	11.3 ± 2.5	19.5 ± 2.1	19.7 ± 6.4	99.3 ± 20.4	113.3 ± 8.5	267.3 ± 30.7	313.3 ± 12.2
	1500	7.3 ± 1.5	10.0 ± 4.4	18.3 ± 4.0	22.7 ± 4.0	79.0 ± 9.5	93.3 ± 8.1	324.0 ± 51.4*	366.7 ± 71.8*
	5000	14.0 ± 1.0	10.7 ± 6.7	18.0 ± 1.7	22.7 ± 4.0	109.0 ± 21.9	110.7 ± 21.2	352.0 ± 45.0*	387.3 ± 32.9*
Positive control	(b)	316.7 ± 80.8*	204.7 ± 24.2*	271.3 ± 26.4*	2650.7 ± 547.8*	561.3 ± 74.3*	2800.0 ± 688.0*	874.7 ± 124.0*	869.3 ± 203.9*
	0[a]	5.7 ± 1.6	5.8 ± 2.9	12.8 ± 3.9	22.8 ± 1.8	92.3 ± 9.0	92.8 ± 14.4	150.7 ± 16.5	157,2 ± 13,8
Smoked	50	4.7 ± 0.6	7.7 ± 1.2	13.3 ± 1.5	17.7 ± 0.6	76.7 ± 9.0	95.7 ± 2.9	139.3 ± 17.2	171,3 ± 15,8
roast	150	6.7 ± 2.1	9.7 ± 5.5	12.0 ± 5.3	25.0 ± 7.8	87.0 ± 8.5	82.0 ± 10.4	122.7 ± 7.6	215,3 ± 36,5
chicken	500	4.3 ± 2.1	5.3 ± 1.2	11.0 ± 6.1	27.7 ± 2.9	87.7 ± 10.2	103.7 ± 5.5	144.7 ± 25.5	228,3 ± 24,8*
	1500	9.7 ± 2.1	6.7 ± 4.9	17.7 ± 1.5	30.7 ± 3.8	88.7 ± 2.5	100.3 ± 4.7	182.0 ± 15.1	281,0 ± 36,0*
	5000	9.3 ± 4.7	9.7 ± 3.1	16.7 ± 7.1	31.3 ± 10.1	123.3 ± 8.4*	160.7 ± 19.4*	161.3 ± 34.8	304,0 ± 25,0*
Positive control	(b)	354.0 ± 83.2*	210.7 ± 28.0*	256.0 ± 49.2*	1882.7 ± 24.4*	392.7 ± 41.7*	2805.3 ± 64.7*	1040.0 ± 264.4*	386,7 ± 143,6*
	0[a]	10.2 ± 2.9	16.0 ± 1.7	26.8 ± 4.6	39.8 ± 3.0	98.0 ± 8.3	85.8 ± 13.4	183.7 ± 37.8	230.3 ± 50.7
Smoked	50	9.0 ± 6.1	15.7 ± 4.5	21.7 ± 4.0	33.7 ± 6.8	102.0 ± 9.8	94.3 ± 14.3	220.7 ± 45.0	291.3 ± 38.1
roast beef	150	10.7 ± 3.5	17.0 ± 6.0	28.3 ± 6.1	37.7 ± 2.1	125.3 ± 3.1*	109.3 ± 8.5	251.3 ± 41.3	393.3 ± 41.0*
	500	11.3 ± 2.1	15.0 ± 4.4	26.3 ± 4.2	38.3 ± 4.0	110.7 ± 8.1	114.3 ± 4.2	248.7 ± 41.1	398.7 ± 16.0*
	1500	10.3 ± 4.0	17.3 ± 3.5	22.0 ± 4.4	38.0 ± 5.3	99.3 ± 6.5	114.0 ± 10.0	356.0 ± 12.0*	430.0 ± 44.5*
	5000	12.7 ± 1.5	12.3 ± 1.5	35.5 ± 10.6	39.3 ± 4.0	118.0 ± 1.7*	130.3 ± 15.5*	336.7 ± 29.1*	447.3 ± 76.8*
Positive control	(b)	609.7 ± 112.1*	320.0 ± 83.8*	501.3 ± 30.3*	1488.0 ± 226.8*	661.3 ± 33.3*	2501.3 ± 563.7*	1085.3 ± 266.6*	516.0 ± 34.2*
	0[a]	7.0 ± 2.3	8.5 ± 4.3	16.3 ± 4.4	27.5 ± 1.9	89.0 ± 5.6	84.3 ± 6.7	164.7 ± 36.1	185.0 ± 26.2
Chicken	50	6.7 ± 2.1	7.7 ± 1.2	15.0 ± 6.0	21.0 ± 3.5	124.0 ± 15.1*	84.0 ± 10.0	212.7 ± 26.4	183.3 ± 52.6
chawarma	150	5.7 ± 2.1	9.0 ± 5.0	14.0 ± 5.6	23.7 ± 5.9	88.7 ± 9.2	110.7 ± 19.2	218.0 ± 37.4	236.7 ± 32.4
	500	7.3 ± 1.2	3.7 ± 2.1	19.0 ± 3.5	23.0 ± 4.4	92.0 ± 12.5	102.7 ± 19.7	256.7 ± 42.9*	233.3 ± 19.0
	1500	11.3 ± 3.1	7.0 ± 1.7	15.0 ± 5.2	32.0 ± 8.5	144.0 ± 10.6*	113.3 ± 5.0	322.7 ± 27.6*	287.3 ± 18.1*
	5000	7.7 ± 5.1	5.7 ± 1.5	21.3 ± 3.8	36.3 ± 9.9	154.7 ± 28.4*	170.7 ± 24.0*	384.0 ± 33.4*	312.0 ± 30.2*
Positive control	(b)	542.7 ± 69.9*	235.3 ± 28.9*	245.3 ± 53.7*	2378.7 ± 536.7*	432.0 ± 34.9*	2741.3 ± 320.5*	546.7 ± 56.9*	477.3 ± 56.9*

	0[a]	10.2 ± 2.9	16.0 ± 1.7	26.8 ± 4.6	39.8 ± 3.0	98.0 ± 8.3	85.8 ± 13.4	183.7 ± 37.8	230.3 ± 50.7
Beef	50	12.3 ± 1.5	13.0 ± 1.7	27.3 ± 5.7	47.0 ± 3.6	100.7 ± 9.1	94.3 ± 22.2	163.3 ± 3.1	202.7 ± 6.1
jambon	150	12.3 ± 4.7	14.7 ± 5.1	30.3 ± 6.1	44.3 ± 12.3	127.3 ± 8.1*	108.0 ± 9.6	207.3 ± 26.6	282.7 ± 14.2
	500	6.3 ± 3.2	12.7 ± 3.2	35.3 ± 6.8	47.0 ± 7.0	109.3 ± 6.7	117.3 ± 14.8	261.3 ± 44.4	287.3 ± 15.5
	1500	13.3 ± 3.1	14.0 ± 2.6	36.3 ± 2.5	49.3 ± 9.8	113.7 ± 8.1	110.0 ± 7.5	254.7 ± 30.3	357.3 ± 23.2*
	5000	12.0 ± 2.6	13.7 ± 4.0	47.0 ± 10.6*	58.0 ± 14.7	157.0 ± 20.7*	145.3 ± 11.8*	212.7 ± 21.2	402.7 ± 11.0*
Positive control	(b)	609.7 ± 112.1*	320.0 ± 83.8*	501.3 ± 30.3*	1488.0 ± 226.8*	661.3 ± 33.3*	2501.3 ± 563.7*	1085.3 ± 266.6*	516.0 ± 34.2*

[a] DMSO; ov: Overgrown revertants; : Biologically significant

[*] Statistically significant difference compared with the negative control ($p < 0.01$);

[b] TA100 (- S9): Sodium azide (1); TA1537 (- S9): 9-amino-acridine (50); TA98 (- S9): 2-nitrofluorene (2); TA102 (- S9): Mitomycin C (0.125).

TA1537, TA98, TA100 (+ S9): 2-anthramine (2); TA102 (+ S9): benzo(a)pyrene (2).

Table III. Mutagenic activity of hexane extracts of industrially processed meat products.

Hexane extracts	Doses µg/plate	Number of revertant colonies (Mean ± standard deviation (SD))							
		TA1537		TA98		TA100		TA102	
		- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
Corned-beef made in Algeria	0[a]	6.2 ± 2.8	7.5 ± 3.5	19.5 ± 6.1	23.5 ± 7.0	86.8 ± 12.8	83.0 ± 7.2	144.2 ± 32.6	170.0 ± 30.3
	10	7.7 ± 1.2	7.7 ± 1.5	24.7 ± 6.4	28.3 ± 4.2	99.7 ± 10.1	91.3 ± 7.6	145.3 ± 11.6	210.7 ± 42.3
	30	7.7 ± 2.1	8.3 ± 3.1	22.7 ± 3.2	30.0 ± 9.8	116.3 ± 4.2	85.0 ± 16.1	151.3 ± 11.0	264.0 ± 6.9*
	100	8.3 ± 3.8	6.3 ± 2.5	23.0 ± 3.0	30.3 ± 7.5	95.0 ± 13.9	93.0 ± 6.9	172.7 ± 19.6	228.0 ± 34.0
	300	5.0 ± 1.7	4.7 ± 1.5	20.3 ± 2.5	27.3 ± 7.0	110.7 ± 12.4	93.3 ± 12.1	183.7 ± 6.7	230.7 ± 29.7
	1000	6.0 ± 3.6	5.7 ± 1.5	20.0 ± 7.5	30.3 ± 4.0	96.7 ± 9.5	81.7 ± 10.2	166.3 ± 38.4	221.3 ± 31.6
	Positive control (b)	423.3 ± 45.0*	330.0 ± 35.4*	250.7 ± 32.1*	2640.0 ± 267.3*	393.3 ± 78.4*	2560.7 ± 393.8*	857.3 ± 173.0*	838.7 ± 81.2*
Imported corned-beef	0[a]	8.8 ± 4.5	6.7 ± 0.8	24.5 ± 5.0	32.5 ± 6.0	76.7 ± 9.5	70.2 ± 9.8	81.0 ± 23.5	173.7 ± 38.3
	10	7.0 ± 2.0	4.7 ± 2.1	24.3 ± 0.6	22.3 ± 4.7	75.0 ± 15.1	81.7 ± 10.7	114.0 ± 39.9	206.7 ± 15.0
	30	9.3 ± 3.1	5.7 ± 2.5	17.3 ± 3.8	35.3 ± 6.1	77.3 ± 10.2	61.3 ± 5.5	149.0 ± 46.1	180.7 ± 35.6
	100	7.7 ± 2.1	6.0 ± 2.0	18.3 ± 5.5	22.0 ± 3.6	87.3 ± 2.5	73.0 ± 15.7	108.0 ± 16.6	169.3 ± 32.9
	300	8.3 ± 6.0	4.0 ± 3.5	19.0 ± 6.1	28.0 ± 6.1	86.7 ± 7.6	78.7 ± 5.9	166.0 ± 23.6*	194.7 ± 27.0
	1000	7.7 ± 2.9	6.0 ± 1.7	22.7 ± 7.5	27.3 ± 3.5	91.3 ±8.1	71.7 ± 4.2	150.3 ± 12.2	128.7 ± 20.5
	Positive control (b)	618.7 ± 211.3*	528.0 ± 42.3*	258.0 ± 65.8*	3234.3 ± 882.1*	661.3 ± 24.4*	1568.0 ± 112.0*	1333.3 ± 60.6*	738.7 ± 192.1*
Salami	0[a]	7.8 ± 1.7	10.0 ± 2.8	25.8 ± 3.1	35.0 ± 4.0	81.5 ± 8.5	60.7 ± 4.4	109.8 ± 26.7	162.7 ± 15.9
	10	11.0 ± 4.0	8.0 ± 2.0	27.3 ± 6.7	26.7 ± 1.5	89.7 ± 3.8	69.7 ± 6.7	104.7 ± 9.5	204.0 ± 7.2*
	30	8.0 ± 2.0	13.7 ± 1.2	30.0 ± 3.0	26.7 ± 2.1	94.7 ± 3.2	77.3 ± 16.0	127.3 ± 19.7	234.0 ± 9.2*
	100	9.3 ± 3.2	8.3 ± 3.5	27.3 ± 3.2	27.3 ± 2.1	87.3 ± 15.9	86.0 ± 13.5*	134.0 ± 7.2	244.7 ± 33.8*
	300	8.0 ± 3.0	6.0 ± 2.6	32.7 ± 3.1	28.3 ± 2.9	85.3 ± 15.5	79.3 ± 13.7	125.3 ± 18.1	202.7 ± 10.3
	1000	9.3 ± 0.6	10.0 ± 3.6	24.0 ± 7.9	33.3 ± 3.5	95.3 ± 9.1	74.7 ± 0.6	96.7 ± 18.9	212.7 ± 15.0*
	Positive control (b)	294.0 ± 80.2*	201.3 ± 46.4*	294.0 ± 66.8*	1098.7 ± 88.1*	538.0 ± 53.9*	1141.3 ± 148.7*	654.7 ± 53.7*	634.7 ± 28.1*

	0[a]	7.8 ± 1.7	10.0 ± 2.8	25.8 ± 3.1	35.0 ± 4.0	81.5 ± 8.5	60.7 ± 4.4	109.8 ± 26.7	162.7 ± 15.9
K-chir	10	9.7 ± 3.8	16.7 ± 3.8	32.3 ± 4.6	36.0 ± 1.0	95.3 ± 10.0	83.3 ± 7.4	125.7 ± 33.5	261.3 ± 61.0
	30	8.7 ± 3.8	15.7 ± 2.9	29.7 ± 4.5	37.0 ± 2.0	96.7 ± 5.0	88.7 ± 17.0	186.0 ± 14.1*	336.7 ± 77.0*
	100	8.7 ± 3.2	16.0 ± 1.7	26.7 ± 1.2	33.3 ± 3.2	100.0 ± 7.5	89.7 ± 19.6	190.3 ± 31.1*	348.0 ± 45.8*
	300	10.3 ± 3.2	11.3 ± 1.5	32.0 ± 2.0	35.7 ± 3.8	90.0 ± 8.7	98.3 ± 18.2*	211.3 ± 25.7*	380.0 ± 48.9*
	1000	10.3 ± 1.2	13.7 ± 4.0	29.3 ± 4.5	36.0 ± 2.6	105.7 ± 9.0*	89.0 ± 1.0	141.7 ± 37.2	265.3 ± 41.1
Positive control	(b)	294.0 ± 80.2*	201.3 ± 46.4*	294.0 ± 66.8*	1098.7 ± 88.1*	538.0 ± 53.9*	1141.3 ± 148.7*	654.7 ± 53.7*	634.7 ± 28.1*
	0[a]	3.7 ± 1.2	6.7 ± 2.4	17.0 ± 4.5	27.3 ± 6.1	84.7 ± 7.5	67.3 ± 14.2	90.2 ± 13.1	177.7 ± 26.1
Smoked	10	8.7 ± 0.6	7.0 ± 3.6	23.3 ± 3.1	29.0 ± 6.1	86.7 ± 16.8	75.3 ± 5.5	108.7 ± 9.5	145.7 ± 19.6
roast	30	9.0 ± 6.6	10.7 ± 4.0	18.7 ± 4.7	29.0 ± 4.6	90.0 ± 10.4	93.3 ± 23.2	126.0 ± 23.6	226.7 ± 56.0
chicken	100	6.7 ± 0.6	8.0 ± 1.0	17.0 ± 6.6	35.0 ± 4.0	85.7 ± 29.7	91.3 ± 10.7	111.3 ± 18.1	233.3 ± 27.7
	300	7.3 ± 3.1	6.7 ± 2.5	19.0 ± 2.6	34.7 ± 7.5	95.3 ± 4.7	85.3 ± 7.5	114.7 ± 18.5	247.3 ± 18.1
	1000	9.7 ± 0.6	7.7 ± 6.0	19.3 ± 2.9	28.7 ± 8.1	80.3 ± 11.2	61.7 ± 24.0	93.3 ± 8.1	144.7 ± 64.7
Positive control	(b)	453.3 ± 126.0*	248.0 ± 4.0*	236.7 ± 51.3*	2848.0 ± 306.5*	474.7 ± 18.1*	1492.0 ± 250.0*	452.7 ± 33.2*	412.0 ± 62.1*
	0[a]	6.3 ± 1.6	7.7 ± 1.8	20.3 ± 2.5	24.0 ± 6.7	79.8 ± 6.8	72.3 ± 15.2	121.3 ± 31.2	159.0 ± 33.9
Smoked	10	5.0 ± 3.0	5.7 ± 2.1	18.3 ± 4.2	27.7 ± 2.3	70.3 ± 9.1	75.7 ± 13.9	122.0 ± 10.6	166.7 ± 18.6
roast beef	30	6.3 ± 2.1	5.3 ± 3.8	14.0 ± 3.6	23.0 ± 1.0	78.0 ± 14.8	72.3 ± 9.5	132.0 ± 13.9	210.7 ± 25.3
	100	7.3 ± 4.5	5.7 ± 0.6	20.7 ± 7.2	22.7 ± 5.9	76.3 ± 3.8	70.7 ± 3.5	140.0 ± 27.1	190.0 ± 20.0
	300	2.7 ± 1.5	7.0 ± 3.0	20.3 ± 9.0	25.7 ± 1.5	68.0 ± 13.0	68.7 ± 7.5	130.0 ± 14.0	202.7 ± 17.5
	1000	4.0 ± 3.6	5.0 ± 1.0	19.0 ± 2.0	31.0 ± 6.6	60.7 ± 5.9	66.3 ± 5.7	120.0 ± 12.2	179.3 ± 20.5
Positive control	(b)	249.3 ± 9.0*	405.3 ± 52.8*	202.7 ± 20.0*	2240.0 ± 284.4*	348.0 ± 29.5*	2944.0 ± 461.2*	830.7 ± 237.7*	374.7 ± 58.0*
	0[a]	3.7 ± 1.2	6.7 ± 2.4	17.0 ± 4.5	27.3 ± 6.1	84.7 ± 7.5	67.3 ± 14.2	90.2 ± 13.1	177.7 ± 26.1
Chicken	10	5.0 ± 2.0	9.0 ± 1.0	21.3 ± 4.0	19.7 ± 1.2	95.7 ± 27.2	74.7 ± 16.0	98.7 ± 20.4	174.0 ± 22.5
chawarma	30	10.3 ± 1.5*	8.7 ± 2.1	21.3 ± 6.4	22.0 ± 3.5	99.0 ± 12.3	95.0 ± 4.6	116.0 ± 7.2	158.0 ± 19.3
	100	4.3 ± 3.2	9.0 ± 4.0	17.3 ± 0.6	16.0 ± 2.6*	84.3 ± 11.2	87.3 ± 10.7	111.3 ± 9.0	193.3 ± 7.0
	300	7.3 ± 2.3	8.7 ± 4.6	21.3 ± 9.1	15.7 ± 0.6*	88.7 ± 7.4	87.7 ± 9.0	114.7 ± 12.7	221.3 ± 67.0
	1000	4.3 ± 0.6	8.3 ± 0.6	23.3 ± 4.2	17.0 ± 3.6*	95.7 ± 15.6	89.0 ± 22.5	60.0 ± 62.5	180.7 ± 6.1
Positive control	(b)	453.3 ± 126.0*	248.0 ± 4.0*	236.7 ± 51.3*	2848.0 ± 306.5*	474.7 ± 18.1*	1492.0 ± 250.0*	452.7 ± 33.2*	412.0 ± 62.1*

	0[a]	6.3 ± 1.6	7.7 ± 1.8	20.3 ± 2.5	25.7 ± 7.3	79.8 ± 6.8	72.3 ± 15.2	99.7 ± 49.0	159.0 ± 33.9
Beef	10	8.0 ± 2.6	7.3 ± 1.5	29.3 ± 2.5	30.7 ± 8.4	89.7 ± 12.7	73.0 ± 10.4	114.0 ± 4.0	220.0 ± 30.3
jambon	30	5.3 ± 1.5	8.3 ± 0.6	26.3 ± 2.5	34.0 ± 4.4	92.3 ± 19.6	96.0 ± 13.0	185.3 ± 34.2	225.3 ± 11.0
	100	7.3 ± 2.5	7.0 ± 3.6	22.7 ± 8.6	42.7 ± 7.6	110.3 ± 12.1	100.3 ± 5.9	188.7 ± 27.3	244.7 ± 27.3*
	300	7.0 ± 2.0	11.0 ± 4.4	24.3 ± 4.5	36.3 ± 4.5	105.3 ± 17.6	109.3 ± 27.2	197.3 ± 9.2	310.7 ± 11.0*
	1000	6.7 ± 2.1	7.7 ± 0.6	21.0 ± 5.3	36.0 ± 19.3	94.7 ± 16.8	95.7 ± 4.5	166.0 ± 26.2	217.7 ± 26.6
Positive control	(b)	249.3 ± 9.0*	405.3 ± 52.8*	202.7 ± 20.0*	2240.0 ± 284.4*	348.0 ± 29.5*	2944.0 ± 461.2*	830.7 ± 237.7*	374.7 ± 58.0*

[a] ethanol; Biologically significant

[*] Statistically significant difference compared with the negative control;

[b] TA100 (- S9): Sodium azide (1); TA1537 (- S9): 9-amino-acridine (50); TA98 (- S9): 2-nitrofluorene (2); TA102 (- S9): Mitomycin C (0.125).

TA1537, TA98, TA100 (+ S9): 2-anthramine (2); TA102 (+ S9): benzo(a)pyrene (2).

For corned-beef made in Algeria and beef jambon extracts, methanol and n-hexane extracts induced no biologically significant changes in the mean number of revertants (induction ratio higher than 2) in the four *S. typhimurium* strains TA1537, TA98, TA100 and TA102, either in the absence or in the presence of metabolic activation system. In contrast, a statistically significant increases in the mean number of revertants were noted for methanol extracts in TA98, TA100 and TA102 strains in the absence and / or presence of S9 fraction and for hexane extracts in TA102 strain in the presence of S9 fraction.

The methanol extract of imported corned-beef induced a biologically and statistically significant increases in the mean number of revertants in strain TA98 at the highest dose of 5000 µg/plate, and in strain TA102 at the two highest doses of 1500 and 5000 µg/plate in the presence of S9, and a statistically but not biologically significant increases in strains TA100 and TA102 in the absence and presence of S9. As for hexane extract, in strain TA102, a statistically significant response was observed at the dose of 300 µg/plate in the absence of S9.

For salami extracts, the methanol extract induced also a biologically and statistically significant increases in the mean number of revertants in strain TA100 at the dose of 5000 µg/plate in the absence of S9 and in strain TA102 at the dose of 1500 µg/plate in the presence of S9. Furthermore, a statistically but not biologically significant increases in the mean number of revertants were noted in TA100 and TA102 strains for both methanol and hexane extracts.

For K-chir extracts, biologically significant responses were observed, for hexane extract, in strain TA102 at the doses of 30, 100 and 300 µg/plate in the presence of metabolic activation, with a clear dose–response relationship. Statistically significant responses were also observed in strains TA100 and / or TA102 for both methanol and hexane extracts.

For smoked roast chicken and smoked roast beef extracts, the methanol extracts showed statistically significant increases in the mean number of revertants in strains TA100 and TA102. However, for hexane extracts, neither a biologically nor a statistically significant increase in the mean number of revertants was noted in the four *S.typhimurium* strains TA1537, TA98, TA100 and TA102.

Finally, for chicken chawarma extracts, the methanol extract showed biologically and statistically significant increases in the number of revertants in strains TA100 and TA102. In return, for hexane extract, a statistically significant increase in the mean number of revertants was observed in strain TA98 at the three highest doses of 100, 300 and 1000 µg/plate in the presence of S9 fraction.

No signals of toxicity and/or precipitation were observed during the test performance.

3.2 Treat-and-wash assay

Table IV shows the results of the mutagenic potential using the treat-and-wash assay for the industrially processed meat extracts, which produced an induction ratio higher than 2 in the standard plate incorporation assay.

Table IV. Number of revertants in the treat-and-wash assay generated by the different concentrations of sample extracts showing biologically significant in the standard plate incorporation assay.

Sample extracts	Doses µg/plate	Number of revertant colonies (Mean ± standard deviation (SD))				
		TA98	TA100		TA102	
		+ S9	- S9	+ S9	- S9	+ S9
	0 [a]	31.3 ± 10.7	-	-	-	203.7 ± 46.7
Imported	50	36.0 ± 7.5	-	-	-	172.7 ± 16.8
corned-beef	150	39.0 ± 1.4	-	-	-	197.3 ± 30.1
(methanol	500	33.3 ± 9.5	-	-	-	277.3 ± 8.1
extract)	1500	35.3 ± 5.7	-	-	-	318.7 ± 12.9*
	2500	38.0 ± 6.1	-	-	-	320.7 ± 18.9*
Positive control	(c)	990.7 ± 135.3*	-	-	-	628.0 ± 58.9*
	0 [a]	-	142.0 ± 6.7	-	-	203.7 ± 46.7
Salami	50	-	105.3 ± 23.2	-	-	226.7 ± 27.0
(methanol	150	-	144.0 ± 19.7	-	-	261.3 ± 23.4
extract)	500	-	145.0 ± 23.5	-	-	263.3 ± 31.0
	1500	-	131.0 ± 39.6	-	-	310.7 ± 21.4*
	2500	-	122.0 ± 36.4	-	-	283.3 ± 50.0
Positive control	(c)	-	2885.3 ± 1257.9*	-	-	628.0 ± 58.9*
	0 [a]	-	-	114.5 ± 15.6	208.3 ± 23.6	-
Chicken	50	-	-	125.3 ± 1.2	226.0 ± 22.5	-
chawarma	150	-	-	131.7 ± 1.5	230.0 ± 16.4	-
(methanol	500	-	-	146.0 ± 16.1	215.7 ± 43.0	-
extract)	1500	-	-	143.3 ± 9.1	238.7 ± 36.5	-
	2500	-	-	122.3 ± 18.9	209.0 ± 1.0	-
Positive control	(c)	-	-	1968.0 ± 180.3*	1050.7 ± 73.9*	-
	0 [b]	-	-	-	-	192.3 ± 24.3
K-chir (hexane	10	-	-	-	-	218.7 ± 41.2
extract)	30	-	-	-	-	279.3 ± 14.7*
	100	-	-	-	-	272.7 ± 4.2

	200	-	-	-	-	236.7 ± 46.6
	250	-	-	-	-	283.3 ± 21.4*
Positive control	(c)	-	-	-	-	757.3 ± 89.2*

[a] DMSO; [b] ethanol;

[*] Significantly different from the negative control at $p < 0.01$;

[c] TA98 and TA100 (+ S9): benzo (a) pyrene (15); TA100 (- S9): 4NQO (0.5); TA102 (- S9): Mitomycin C (1); TA102 (+ S9): benzo (a) pyrene (20).

No biologically and / or statistically significant changes in the mean number of revertants in strain TA98 for methanol extract of imported corned-beef, in strains TA100 and TA102 for methanol extract of chicken chawarma and in strain TA100 for methanol extract of salami. In return, statistically but not biologically significant changes in the mean number of revertants were noted in strain TA102 for methanol extract of imported corned-beef at the two highest doses of 1500 and 2500 µg/plate, for methanol extract of salami at the dose of 1500 µg/plate and for hexane extract of K-chir at the doses of 30 and 250 µg/plate. Indeed, the data highlight clear negative results in the modified Ames test. Taking into account the nature of test items, it can be assumed that the significant effects observed in the main trials are probably due to the presence of amino acid (histidine), peptides and / or proteins in the extracts.

3.3 *In vitro* micronucleus assay in micromethod

The results of the *in vitro* micronucleus assay of industrially processed meat extracts are demonstrated in Tables V and VI.

Table V. *In vitro* micronucleus assay results in TK6 human lymphoblastoid cells following treatment with methanol extracts of processed meat.

Methanol extracts	Doses µg/mL	Without S9-mix				With S9-mix	
		3 h short treatment with a 24 h recovery period		27 h continuous treatment without recovery period		3 h short treatment with a 24 h recovery period	
		% RS	MMNC/ 2000 MNC	% RS	MMNC/ 2000 MNC	% RS	MMNC/ 2000 MNC
Corned-beef made in Algeria	0	100	5	100	7	100	7
	125	93.7	3	114.2	4	96.0	8
	250	101.4	7	110.9	5	95.2	8
	500	106.2	6	111.4	4	96.4	11
Imported corned-beef	0	100	5	100	7	100	7
	125	96.7	7	113.4	9	101.1	5
	250	94.7	3	110.2	12	104.2	6
	500	99.9	3	110.7	10	107.6	7
Salami	0	100	5	100	7	100	7
	125	97.8	3	109.9	7	99.7	7
	250	96.7	4	104.3	6	102.7	10
	500	99.9	6	109.9	12	107.4	9
K-chir	0	100	5	100	7	100	7
	125	103.6	4	111.5	11	108.6	11
	250	98.0	4	102.5	4	113.9	10
	500	103.1	6	108.7	4	110.4	8
Smoked roast chicken	0	100	9	100	5	100	5
	125	107.8	12	108.3	7	105.6	12
	250	101.8	5	108.3	9	101.3	4
	500	105.6	9	104.2	10	106.5	11
Smoked roast beef	0	100	9	100	5	100	5
	125	101.3	4	103.2	13	107.1	3
	250	94.4	7	97.8	9	98.8	8
	500	103.8	5	103.9	7	105.6	8

Chicken chawarma	0 125 250 500	100 101.6 97.5 105.6	9 9 3 5	100 106.7 106.2 110.9	5 5 7 11	100 106.5 105.6 109.0	5 16* 4 8
Beef jambon	0 125 250 500	100 103.4 94.9 103.4	9 4 6 13	100 112.2 101.5 105.8	5 8 8 6	100 106.5 96.8 109.2	5 7 12 3
Mitomycin C Mitomycin C Griseofulvin Cyclophosphamide	0.5 0.2 5 5	64.9 - - -	105** - - -	- 64.3 66.8 -	- 81** 45** -	- - - 91.4	- - - 47**

% RS: percentage of relative survival/negative control; MMNC: micronucleated mononucleated cells; MNC: mononucleated cells;

Threshold of statistical significance as compared with the negative control using the CHI2 test;

*$p < 0.05$.

**$p < 0.01$.

Table VI. *In vitro* micronucleus assay results in TK6 human lymphoblastoid cells following treatment with hexane extracts of processed meat.

Hexane extracts	Doses µg/mL	Without S9-mix				With S9-mix	
		3 h short treatment with a 24 h recovery period		27 h continuous treatment without recovery period		3 h short treatment with a 24 h recovery period	
		% RS	MMNC/ 2000 MNC	% RS	MMNC/ 2000 MNC	% RS	MMNC/ 2000 MNC
Corned-beef made in Algeria	0 25 50 100	100 92.2 90.8 93.7	8 8 1* 11	100 95.0 96.3 97.2	10 9 10 20	100 92.8 92.2 103.4	14 5* 10 12
Imported corned-beef	0 25 50 100	100 86.0 93.5 91.8	8 4 13 11	100 96.8 103.2 109.1	10 11 10 11	100 98.7 104.0 108.0	14 9 10 8
Salami	0 25 50 100	100 84.5 89.8 90.2	8 13 9 6	100 97.6 98.8 107.8	10 10 10 6	100 102.4 104.8 110.9	14 7 11 11
K-chir	0 25 50 100	100 89.2 88.2 94.0	8 4 7 8	100 89.9 91.9 94.3	10 8 12 10	100 105.0 102.4 116.3	14 6 4* 5*
Smoked roast chicken	0 25 50 100	100 104.1 102.4 102.7	5 7 7 10	100 98.2 95.4 95.5	7 8 9 9	100 94.5 99.0 97.2	6 2 5 3

Smoked roast beef	0 25 50 100	100 99.5 89.0 104.5	5 10 8 10	100 101.0 96.2 90.5	7 11 13 13	100 103.5 100.0 105.1	6 4 3 3
Chicken chawarma	0 25 50 100	100 95.7 96.1 104.3	5 4 4 2	100 96.6 96.6 97.9	7 3 8 8	100 102.0 103.2 99.0	6 1 12 2
Beef jambon	0 25 50 100	100 98.9 92.0 100.3	5 6 5 6	100 104.8 97.6 95.9	7 5 3 4	100 103.5 85.7 102.0	6 2 2 7
Mitomycin C Mitomycin C Griseofulvin Cyclophosphamide	0.5 0.2 5 5	60.6 - - -	87** - - -	- 64.7 67.4 -	- 68** 82** -	- - - 85.5	- - - 39**

% RS: percentage of relative survival/negative control; MMNC: micronucleated mononucleated cells; MNC: mononucleated cells;

Threshold of statistical significance as compared with the negative control using the CHI2 test;

*$p < 0.05$.

**$p < 0.01$.

The values of the cytotoxicity calculated in TK6 human lymphoblastoid cells were above the limit recommended in the OECD guideline No. 487 (value ≥ 55 ± 5%). The positive controls showed a significant increase ($p < 0.001$) in micronucleated cell frequencies in the presence and absence of S9-mix.

For all methanol extracts, no significant effects on the formation of micronuclei in TK6 cells either in the presence or in the absence of metabolic activation were observed except a statistically significant increase in chicken chawarma methanol extract only at the lowest dose of 125 µg/mL in the 3 h treatment followed by 24 h recovery period with S9 fraction.

For hexane extracts, only the corned-beef made in Algeria in the 27 h continuous treatment without metabolic activation showed a biologically significant increase in the number of micronucleated cells at the highest dose of 100 µg/mL, with 20 micronucleated cells / 2000 mononucleated cells versus 10 for the negative control; thus it can be concluded that it has a weak genotoxic effect.

4 Discussion

Industrially processed meat products are widely consumed by the Algerian population especially because of their availability in various flavors and low cost when compared to raw meats (Chikhi and Bencharif, 2016). Thus, it is very important to assure that processed meat products sold in popular supermarkets do not contain substances which might cause risk to consumers' health. In this regard, a conceivable risk is the formation of mutagenic and genotoxic substances during the industrial processing of meats. Screening studies are useful to check that the processes used by meat industry are sound and appropriate from this point of view (Omoruyi and Pohjanvirta, 2014). This study was performed to ascertain the mutagenic and genotoxic potential of industrially processed meat products. For this intention, both the Ames' test and the *in vitro* micronucleus test were carried out to get an overall idea

concerning the effects of these products. Interestingly, this combination of complementary assays allows covering genetic events possibly leading to genotoxicity, *i.e*. gene mutation and structural and numerical chromosomal aberrations.

With regard to the Ames test, our study shows that the majority of the processed meat samples analyzed was non-mutagenic in standard plate incorporation assay in the presence and absence of metabolic activation using four S. *typhimurium* strains TA1537, TA98, TA100 and TA102. The scarce significant increases in the number of colonies obtained with some extracts (methanol extract of imported corned-beef, salami and chicken chawarma; hexane extract of K-chir) are not actually relevant in terms of mutagenicity as no such effect occurred when using the treat and wash methodology. Hence, their initial positive results were probably due to a localized release of histidine, peptides or proteins from sample extracts which lead to false-positive results (Khandoudi et al., 2009; Thompson et al., 2005).

The negative results obtained from smoked roast beef and smoked roast chicken with Ames test may be considered as unexpected. Indeed, it is well-established that the polycyclic aromatic hydrocarbons and the heterocyclic amines are the principal mutagens formed in processed meat during smoking and roasting (Jägerstad and Skog, 2005). A possible explanation for these results is in the processing methods used by the industry such as cooking at low temperatures for short periods of time that can decrease or prevent the formation of mutagens (Knize et al., 1994). Omoruyi and Pohjanvirta (Omoruyi et al., 2014) have been investigated the mutagenicity of industrially processed foods, including processed meat, using a standard Ames assay, treat and wash and methylcellulose overlay assays. They concluded that the true mutagenic potential of pepper salami remains unclear because this product exhibited mutagenic activity when examined in standard plate incorporation assay using TA100 strain, however, it was found mutagenic only in methylcellulose overlay assay, but not in treat and wash assay. In return, they reported that smoked chicken extract was

mutagenic in all three assays (standard Ames assay, treat and wash and methylcellulose overlay assays) with the TA100 strain (with and without metabolic activation). In addition, a mutagenic activity has been found in corned-beef and canned roast beef (Krone and Iwaoka, 1984). In contrast to our outcome, the corned-beef, salami, smoked roast beef and smoked roast chicken were non-mutagenic. This difference in the results may be due to the difference in the ingredients and the processing methods of these products on one hand and the difference in the extraction methods and the range of concentrations assayed on the other hand.

As no single assay is capable to detect all types of potential human mutations with 100% prediction or accuracy (Pathak et al., 2018), the complementary *in vitro* micronucleus assay was carried out. Indeed, Kirkland et al. (Kirkland et al., 2011) concluded that the *in vitro* genotoxicity battery including only two tests "Ames and micronucleus" is sufficient to detect all relevant *in vivo* genotoxins and *in vivo* carcinogens. Moreover, the implementation of these 2 complementary tests is in line with the recommendations of EFSA for food and feed safety assessment ("Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment," 2011). In the present work, most of the products analyzed (87.5 %) induced no chromosomal aberration in the presence or absence of S9-mix as indicated in the *in vitro* micronucleus assay performed on human lymphoblastoid TK6 cells. In return, this investigation shows that the non-polar extract of corned-beef made in Algeria produces a weak genotoxic effect. Due to the complexity of this extract, no exact identification of the agent(s) responsible for the genotoxic activity can be made at the present time, and the source of the genotoxicity will require further investigation. In the case of industrially processed meat products, no more data are available so far about their genotoxicity especially by using the micronucleus test. To the best of our knowledge, this is the first investigation on

genotoxicity of these products using *in vitro* micronucleus assay on TK6 human lymphoblastoid cells.

Overall, our results showed that no mutagenic potential was found whatever the industrially processed meat products tested except for one of these products that induced a weak genotoxic effect. Consequently, the present study demonstrates the importance of performing dietary monitoring of processed foods using genotoxicity tests to evaluate their potential harmful effects on consumers. However, further in vitro investigations regarding the genotoxic effect of processed meat could be implemented on gastro-intestinal tract target cells (e.g., Human colon T84 or Caco-2 cells). Moreover, more investigations need to be performed with an accurate analytical approach to clear up the exact identity of the chemical compound(s) from complex mixture responsible for genotoxic activity. This task will be necessary to determine the sources of toxic contaminants.

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6 Conflict of interest

The authors declare that there are no conflicts of interest.

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