Université du Québec

INRS – Institut Armand-Frappier

Effect of the addition of various additives on the irradiation sensitivity of *Escherichia coli* and *Salmonella typhi* present in ground beef

Par

Francine Chiasson

Mémoire présenté

pour l'obtention
du grade de Maître ès sciences (M. Sc.)
en Microbiologie appliquée

Jury d'évaluation

Présidente du jury et Examinatrice interne	Darakhshan Ahmad, INRS – IAF
Examinateur externe	Joseph Borsa, MDS Nordion
Directrice de recherche	Monique Lacroix, INRS – IAF

Table of content

List of tables

Tableau 28a Compte bactérien normalisé (log UFC/g) de *S. typhi* dans le bœuf haché en présence de la combinaison de carvacrol (1.0 %) avec du tétrasodium pyrophosphate (0.1 %) à deux températures (4°C and –80°C) à différentes doses d'irradiation pour la détermination de la D_{10}

List of figures

Abstract

Ground beef is an easily perishable food product. It is linked to several cases of food poisoning. Several foodborne pathogens can be found at a very high concentration. One way to assure the innocuity and to improve the bacterial quality of ground beef is the use of irradiation. Since December 1997, the United States of America has approved the use of irradiation to control or eliminated pathogenic bacteria.

This research was aimed to evaluate the effect of several additives on the irradiation sensitivity of *E. coli* and *S. typhi*. The goal was to find additives that would have a synergical effect with the irradiation treatment. The irradiation sensitivity (D_{10}) of each bacteria was determined in the presence of each active compound. Following that, the best combination of additives was determined. Also, the irradiation sensitivity of each bacteria in ground beef was determined in the presence of the best combination of additives packed under different temperature and atmospheres.

Results from this study demonstrated that the best active compound was trans-cinnamaldehyde (at a concentration of 1.5 % for *E. coli* and 0.89 % for *S. typhi*), with a D_{10} of 0.037 kGy for *E. coli* and of 0.139 kGy for *S. typhi*. Effectiveness of other additives in decreasing order were : carvacrol (0.88 % for *E. coli* and 1.15 % for *S. typhi*), thymol (1.14 % for *E. coli* and 1.60 % for *S. typhi*) and thyme (2.33 % for *E. coli* and 2.75 % for *S. typhi*), where the D_{10} measured were 0.103 kGy, 0.087 kGy and 0.090 kGy respectively for *E. coli* and 0.208 kGy, 0.210 kGy and 0.260 kGy respectively for *S. typhi*. Not all the additives tested increased the irradiation sensitivity of *E. coli* and *S. typhi*. Addition of ascorbic acid increased the irradiation resistance of *E coli* with a D_{10} value of 0.141 kGy compared to 0.126 kGy for the control, and had no effect on *S. typhi* (D_{10} : 0.521kGy). Thus, of the nine additives tested, carvacrol, ascorbic acid and tetrasodium pyrophosphate were selected to be the best. Trans-cinnamaldehyde was not selected for its inappropriate odour.

Results also indicated that the best bioactive additives were carvacrol alone or in combination with tetrasodium pyrophosphate. The D_{10} were respectively 0.057 kGy and 0.057 kGy for *E. coli*. For *S. typhi*, the D_{10} with carvacrol and the mixture of carvacrol (1.0 %) and tetrasodium pyrophosphate (0.1 %) were respectively 0.235 kGy and 0.254 kGy. The mixture of carvacrol and tetrasodium pyrophosphate was used for the determination of the irradiation sensitivity under various atmospheres and irradiation temperatures. The best results were obtained under MAP (60 % O_2 – 30 % CO_2 – 10 % N_2) in the presence of the mixture of additives, with D_{10} of 0.046 kGy for *E. coli* and 0.053 kGy for *S. typhi* respectively. Under frozen conditions, both *E. coli* and *S. typhi* were more resistant to irradiation than under refrigerated condition, as the D_{10} under frozen conditions increased from 0.055 kGy to 0.128 kGy for *E. coli* and from 0.254 kGy to 0.297 kGy for *S. typhi*.

Résumé long

Le bœuf haché est obtenu à partir de la partie comestible du muscle squelettique d'un animal, qui était en santé au moment de l'abattage (FSIS, 2001). Cette viande est composé d'eau (71 – 73 %), de protéines (20 – 22 %), de lipides (5 – 8 %) et de composés mineurs (moins de 1 %), tels que vitamines, enzymes, composés de pigmentation et composés responsable de la couleur (Lambert, *et al.*, 1991). Le bœuf haché est un aliment avec une durée de conservation variant de 3 à 5 jours, lorsque entreposé à 4°C (Gamage *et al.*, 1997). Cet aliment offre les conditions idéales pour la croissance de bactéries pathogènes, telles que *E. coli* O157:H7, *Salmonella* et *S. aureus*, ainsi que la croissance de bactéries de putréfaction, telles que *Pseudomonas* et *Lactobacillus*.

Une façon d'augmenter la durée de conservation du bœuf haché est l'utilisation de l'irradiation. Il est important de traiter la viande avec une dose d'irradiation minimale permettant la réduction ou l'élimination des pathogènes, sans affecter les propriétés organoleptiques. De plus, la mise au point de technologies permettant la réduction de la dose d'irradiation permettrait de diminuer les coûts et augmenter l'efficacité du traitement. L'ajout de additifs ayant des propriétés antimicrobiennes et/ou antioxydantes à la viande peut avoir l'avantage de créer un effet synergique avec l'irradiation. Parmi les additifs étudiés, plusieurs sont des composés naturels, tel que l'acide ascorbique, l'acide tannique, la carnosine, le carvacrol, la nisine, le romarin, le thym, le thymol et la trans-cinnamaldéhyde. D'autres tel que le BHA, le BHT, l'EDTA et le tétrasodium pyrophosphate sont synthétiques.

Les objectifs de ce travail était de déterminer la sensibilité des bactéries à l'irradiation en présence de chacun des additifs testés, déterminer la meilleure combinaison de additifs sur la sensibilité des bactéries à l'irradiation et de déterminer la sensibilité de chacune des bactéries à l'irradiation en présence de la meilleure combinaison sous différents atmosphère d'emballage et à deux températures différentes.

Du bœuf haché mi-maigre (23 %) irradié à 25 kGy sous forme congelé a été utilisé pour chaque expérience sur la sensibilité de *E. coli* et *S. typhi* à l'irradiation. La sensibilité de chacune des bactéries à l'irradiation a été testée en présence de différentes additifs. Les premières concentrations correspondent à la moyenne des concentrations minimales inhibitrices (CMI) pour réduire d'un log six bactéries pathogènes et de détérioration dans un bouillon de culture. Les moyennes étaient de l'acide ascorbique (0,5 %), l'acide tannique (0,38 %), du carvacrol (0,125 %), du romarin (0,5 %), du thym (0,2 %), du thymol (0,1 %) et de la trans-cinnamaldéhyde (0,025 %). La concentration utilisée pour la carnosine (1,0 %) a été choisie selon la littérature (Sebranek, 1999). La concentration du BHA (0,01 %), BHT (0,01 %), EDTA (100 ppm), tétrasodium pyrophosphate (0,1 %) correspondent aux concentrations recommandées par l'Agence Canadienne en Inspection Alimentaire. Le CMI dans le bœuf haché de cinq additifs a également été étudié. Il s'agit du carvacrol (0,88 % pour *E. coli* et 1,15 % pour *S. typhi*), de la nisine (625 UI/g), du thym (2,33 % pour *E. coli* et 2,75 % pour *S. typhi*), du thymol (1.15 % pour *E. coli* et 1.6 % pour *S. typhi*) et trans-cinnamaldéhyde (1,5 % pour *E. coli* et 0,89 % pour *S. typhi*).

Pour déterminer la sensibilité des bactéries à l'irradiation (D_{10}), il s'agit d'irradier à différentes doses plusieurs échantillons de viande contaminés par l'une ou l'autre des bactéries en présence d'un composé actif. Une fois les échantillons irradiés, ils sont homogénéisés avec de l'eau peptonée stérile et les dilutions appropriées sont incubés dans du TSA (par masse) pendant 24 heures à 35°C.

Avec les résultats obtenus avec l'expérience précédante, trois additifs ont été retenus pour la prochaine étape, soit la détermination de la meilleure combinaison de additifs. Ces trois additifs sont le carvacrol, l'acide ascorbique et le tétrasodium pyrophosphate. La sensibilité de *E. coli* et *S. typhi* à l'irradiation a été déterminée en présence de carvacrol (1,0 %) seul et en combinaison avec l'acide ascorbique(0,5 %) et tétrasodium pyrophosphate (0,1 %).

Par la suite, la sensibilité de chacune des bactéries à l'irradiation a été déterminée en présence de la meilleure combinaison, soit carvacrol en présence de tétrasodium pyrophosphate, sous différentes atmosphères à 4°C (soit air, 100 % CO_2, sous vide et sous MAP : 60 % O_2 - 30 % CO_2 – 10 % N_2). Sous air, la sensibilité a été déterminée à des températures de 4°C et de –80°C.

La détermination de l'oxydation des gras provoqué par l'irradiation du bœuf haché a été déterminé en présence des composés utilisés pour la détermination de la meilleure combinaison et sous les différentes atmosphères étudiées. L'oxydation des lipides a été évalué en déterminant le contenu en TBARS (μM/g) selon la méthode décrite par Giroux (2000) et la courbe standard selon la méthode par Lawlor *et al.* (2000).

Comme expérience préliminaire, CMI de certains additifs a été déterminée dans le bœuf haché en présence de soit *E. coli* ou *S. typhi*. Les trois additifs les plus efficaces pour les deux bactéries étaient le carvacrol, le thymol et la trans-cinnamaldéhyde. Les CMI étaient 0,88 %, 1,14 % et 1,57 % pour *E. coli* et 1,15 %, 1,60 % et 0,89 % pour *S. typhi*, respectivement. Par la suite, il y a eu le thym et le romarin avec des CMI de 2,33 % et 10,37 % pour *E. coli* et de 2,75 % et 13,56 % pour *S. typhi*. Les CMI obtenus en présence de l'acide ascorbique étaient 2,71 % pour *E. coli* et 1.83 % pour *S. typhi*. Finalement, l'ajout de l'acide tannique n'a eu aucun effet inhibiteur sur *E. coli* et sur *S. typhi*, avec des concentration de 11,15 % et 21,18 %, respectivement.

Suite à cette expérience préliminaire, la sensibilité de *E. coli* et de *S. typhi* à l'irradiation a été déterminée en présence des différents additifs. Les résultats obtenus ont démontrés qu'en général, l'ajout de additifs a eu un effet sur la sensibilité de *E. coli* et *S. typhi* à l'irradiation. Les additifs les plus efficaces sont le carvacrol, le thym, le thymol et la trans-cinnamaldéhyde aux concentrations correspondant au CMI.

La D_{10} de *E. coli* en absence de additifs est de 0,126 kGy. Le composé le plus efficace est la trans-cinnamaldéhyde (1,5 %) avec une D_{10} de 0,037 kGy, correspondant à une augmentation en sensibilité à l'irradiation de 70,6 %. Ce composé actif est suivi par le thymol (1,15 %), le thym (2,33 %) et le carvacrol (0,88 %), avec des D_{10} correspondants à 0,087 kGy, 0,090 kGy et 0,103 kGy respectivement. L'efficacité de ces composés à augmenter la sensibilité à l'irradiation

était de 40,0 %, 28,6 % et 18,2 %, respectivement. D'autres additifs ont permis d'augmenter la sensibilité de la bactérie à l'irradiation. Ces additifs sont le thymol (0,1 %), l'acide tannique (0,38 %), le romarin (0,5 %), le BHT (0,01 %), la trans-cinnamaldéhyde (0,025 %), le carvacrol (0,125 %), le thym (0,2 %), le BHA (0,01 %), la nisine (625 UI/g) et le mélange de la nisine (625 UI/g) avec le EDTA (100 ppm). Ces additifs ajoutés aux concentrations identifiées montrent des D_{10} variant de 0,103 kGy à 0,121 kGy. Ceci correspond à une augmentation en sensibilité de 4,0 % à 18,2 %. L'ajout de EDTA, de tétrasodium pyrophosphate et de carnosine n'a eu aucun effet sur la sensibilité de la bactérie. Seulement l'ajout d'acide ascorbique a eu un effet protecteur de 11,9 % sur la bactérie avec une D_{10} de 0,141 kGy comparativement à une D_{10} de 0.126 kGy pour les échantillons sans composé actif ajouté.

Avec les résultats obtenus avec *S. typhi*, il a été possible de déterminer que la D_{10} de la bactérie sans ajout de composé actif était de 0,526 kGy. Tous les additifs étudiés, à l'exception de l'acide ascorbique, ont augmenté la sensibilité de *S. typhi*. Encore une fois, c'est la trans-cinnamaldéhyde qui est la plus efficace avec une D_{10} de 0,139 kGy, correspondant à une efficacité de 73,6 %. Par ordre décroissant, il y a le carvacrol (1,15 %), le thymol (1,6 %) et le thym (2,75 %), avec des D_{10} de 0,208 kGy, 0,210 kGy et 0,260 kGy. L'efficacité des ces trois additifs varie de 60,4 % à 50,6 %. D'autres additifs ont permis d'augmenter la sensibilité de la bactérie à l'irradiation. Ces additifs sont l'acide tannique (0,38 %), le mélange de nisine (625 UI/g) et EDTA (100 ppm), le carvacrol (0,125 %), le tétrasodium pyrophosphate (0,1 %), la trans-cinnamaldéhyde (0,025 %), le thymol (0,1 %), le thym (0,2 %), le BHT (0,01 %), le BHA (0,01 %), l'EDTA (100 ppm), la nisine (625 UI/g), le romarin (0,5 %) et la carnosine (1,0 %). La D_{10} de ces additifs varie de 0,302 kGy à 0,494 kGy, représentant une augmentation en efficacité de 6,1 % à 42,6 %. Seul l'ajout de l'acide ascorbique n'a eu aucun effet sur la sensibilité de la bactérie à l'irradiation

Suite à ces résultats, trois additifs ont été retenus pour déterminer la meilleure combinaison de additifs. Ce sont le carvacrol, pour ses propriétés antimicrobiennes, l'acide ascorbique, pour son habilité à maintenir la couleur de la viande au moment de l'irradiation et le tétrasodium pyrophosphate, pour ses capacités de rétentions d'eau.

Les résultats obtenus démontrent que l'ajout du carvacrol et du mélange de carvacrol et de tétrasodium pyrophosphate permet d'augmenter de plus de 50 % la sensibilité à l'irradiation des deux bactéries. Dans le cas de *E. coli*, la D_{10} diminue de 0,126 kGy pour le témoin à 0,057 kGy en présence du carvacrol et du mélange de carvacrol et de tétrasodium pyrophosphate. La D_{10} de *S. typhi* passe de 0,519 kGy pour le témoin à 0,235 kGy en présence de carvacrol et à 0,254 kGy en présence du mélange de carvacrol et de tétrasodium pyrophosphate.

L'ajout du mélange de carvacrol et acide ascorbique et du mélange de carvacrol, acide ascorbique et de tétrasodium pyrophosphate a permis d'augmenter la sensibilité de *S. typhi*, avec des D_{10} de 0,313 kGy et de 0,344 kGy, respectivement. En présence de *E. coli*, l'ajout du mélange de carvacrol et acide ascorbique n'a eu aucun effet sur la sensibilité de la bactérie, avec une D_{10} de 0,133 kGy. L'ajout du mélange de carvacrol, acide ascorbique et de tétrasodium pyrophosphate a permis d'augmenter la résistance de la bactérie, avec une D_{10} de 0,142 kGy.

Avec ces résultats, il a été déterminé que la meilleure combinaison est le mélange de carvacrol et de tétrasodium pyrophosphate. Cette combinaison a été utilisée pour déterminer la sensibilité à l'irradiation de *E. coli* et *S. typhi* sous différentes atmosphères emballages et températures irradiation.

La sensibilité à l'irradiation de *E. coli* et *S. typhi* a été déterminée sous différentes atmosphères, soit air, CO_2, MAP (modified atmosphere packaging) et sous vide. En général, l'ajout du mélange de additifs aux échantillons a permis d'augmenter la sensibilité des bactéries, quelque soit l'atmosphère d'irradiation (air, CO_2, MAP et sous vide). Les résultats montrent que c'est sous MAP que *E. coli* et *S. typhi* sont les plus sensibles à l'irradiation en absence et en présence du mélange de carvacrol et tétrasodium pyrophosphate. Les D_{10} pour ces deux bactéries étaient de 0,086 kGy et 0,221 kGy en absence du mélange et de 0,046 kGy et de 0,053 kGy en présence du mélange, respectivement. L'utilisation du MAP augmente la sensibilité de *E. coli* de 37,7 % et de 58,0 % pour *S. typhi*.

Sous vide et sous CO_2, en absence du mélange de carvacrol et de tétrasodium pyrophosphate, les D_{10} étaient de 0,118 kGy et 0,123 kGy pour *E. coli* et de 0,420 kGy et 0,429 kGy pour *S. typhi*. Les résultats ont également montré qu'en présence du mélange de carvacrol et de tétrasodium pyrophosphate, *E. coli* et *S. typhi* deviennent plus résistantes à l'irradiation, comparativement à leur témoin respectif. Dans le cas de *E. coli*, les D_{10} étaient de 0,101 kGy sous vide et de 0,106 kGy sous CO_2. Pour *S. typhi*, les D_{10} étaient de 0,308 kGy sous vide et de 0,336 kGy sous CO_2. En absence du mélange de carvacrol et de tétrasodium pyrophosphate, c'est sous air que *E. coli* et *S. typhi* sont les plus résistantes, avec des D_{10} de 0,126 kGy et 0,526 kGy, respectivement.

Suite à cette expérience, la sensibilité de *E. coli* et de *S. typhi* à l'irradiation a été déterminée sous air à deux températures différentes, soit la température à laquelle la viande est irradiée pour la pasteurisation (4°C) et la température à laquelle la viande est irradiée pour la stérilisation (-80°C). Lorsque la viande est sous forme congelée, *E. coli* et *S. typhi* sont beaucoup plus résistantes à l'irradiation que sous forme réfrigérée, et ce, en absence ou en présence du mélange de carvacrol et de tétrasodium pyrophosphate.

En absence du mélange de carvacrol et de tétrasodium pyrophosphate, la D_{10} de *E. coli* passe de 0,126 kGy sous forme réfrigérée à 0,227 kGy sous forme congelée. La D_{10} de *S. typhi* passe de 0,526 kGy sous forme réfrigérée à 0,701 kGy sous forme congelée. Malgré cette résistance accrue sous forme congelés, *E. coli* et *S. typhi* sont tout de même plus sensible en ajoutant le mélange de carvacrol et de tétrasodium pyrophosphate, avec des valeurs de D_{10} de 0,128 kGy et 0,297 kGy, respectivement.

Suite à la détermination des D_{10}, l'oxydation des gras a été déterminée en présence de carvacrol, d'acide ascorbique, de tétrasodium pyrophosphate et de différentes combinaisons de ces additifs dans du bœuf haché irradié et non irradié et emballé sous différentes atmosphères (100 % CO_2; MAP et sous vide). Les résultats démontrent que le mélange de carvacrol (1,0 %) et de tétrasodium pyrophosphate (0,1 %) aidait à stabiliser le contenu en TBARS suite à l'irradiation des échantillons de bœuf haché. Les plus basses valeurs de TBARS ont été observées pour les échantillons de bœuf haché sans composé emballés sous vide (0,977 µM/g pour les échantillons non irradiés vs de 1,373 µM/g pour les échantillons irradiés) et pour les échantillons emballés sous MAP avec les mélanges de additifs (0,808 µM/g pour les échantillons non irradiés vs de 1,138 µM/g pour les échantillons irradiés).

1. Introduction

The ground beef has a shelf life that varies between 3 and 5 days when kept at 4°C (Gamage *et al.* 1997). During the grinding process, the transformation from large cuts to ground product, the contact surface number significantly increases and so are the chances of contamination by bacteria. *Pseudomonas, Enterobacteria* and lactic acid bacteria are responsible for the deterioration of the meat (Roberts and Weese, 1998). Due to this deterioration and depending on the region, one quarter to one third of the production is lost every year world wide (Thakur and Singh, 1994).

This deterioration of the quality is not only responsible for making the meat improper for consumption, but is also responsible for several cases of food poisoning. In the United States, there are about 6 millions cases of food poisoning each year, and the cost associated with it, is evaluated to be in the range of billions of dollars (Radomyski *et al.* 1994). More recently, 77 000 kg of ground beef was recalled in almost all the provinces of Canada due to the presence of *Escherichia coli* O157:H7 (Anonymous, 2000). This means that refrigeration alone is not enough to ensure a meat of good quality and that with all the necessary precautions, there is still a great risk associated with the handling of ground beef. It is therefore important to find a way to reduce the number of bacteria in the meat to be able to ensure a product of better quality.

One way to increase the shelf life is with irradiation of the ground beef at low dose. A decision pronounced in 1980 by the Food and Agriculture Organisation (FAO), International Atomic Energy Agency (IAEA) and World Health Organisation (WHO) has revived the interest in food irradiation (Giroux and Lacroix, 1998). It was determined by the present committee that irradiation at doses lower than 10 kGy had no toxicological effect and causes no nutritional problem. For ground beef, it was demonstrated by Roberts and Weese (1998) that a dose of 3 kGy would increase the shelf life of the ground beef by eliminating up to 99 % of certain pathogens such as *E. coli* O157:H7, *Salmonella*, *Campylobacter jejuni* and *Listeria.*

Further detailed research is necessary to find ways to reduce the irradiation doses used in food product. One possible avenue is a combination of certain antibacterial compounds with irradiation to obtain a synergistic effect. This effect would help to lower the irradiation doses needed to reduce the bacterial population and reduce the cost associated with the treatment of food poisoning. To reach this objective, the synergistic effect of irradiation and the additives on the sensibility of various bacteria in ground beef was to be determined. Nine natural compounds (ascorbic acid, carnosine, carvacrol, nisin, rosemary, tannic acid, thyme, thymol, trans-cinnamaldehyde) and four additives (BHA, BHT, EDTA, tetrasodium pyrophosphate) are tested on *E. coli* and *S. typhi* during this study.

2. Literature Review

Ground beef is a perishable product, since it offers favourable conditions for the growth of microorganisms, including pathogenic and spoilage bacteria. It is a high source of nutrients, water (A_w is between 0.95 and 1.00) and a pH close to neutrality (between 5.3 and 6.00) (Lefebvre *et al.*, 1992). It is therefor not surprising to find a high concentration of pathogenic bacteria like *Escherichia coli* O157:H7, *Salmonella, Staphylococcus aureus* and *Streptococcus*.

Microbial contamination of the meat comes from external sources during bleeding, handling and processing (Frazier, 1967). During bleeding, skinning and cutting, the exterior of the animal, like the skin, hoofs and hair, and the intestinal tract are the main sources of contamination. The bacteria found in meat, may originate from the skin, faecal material or from soil, water and air. It was demonstrated by several researches that low dose of irradiation was an effective way to control the bacterial population in ground beef. Unfortunately, the dosage needed to control the bacterial population will affect some chemical aspects of the ground beef, such as lipids and proteins. These changes may affect the colour and the taste of the meat. It was demonstrated that the addition of ascorbic acid to ground beef before irradiation helps to control the bacterial population without affecting the colour or the taste of the meat at low dose irradiation (Giroux *et al.*, 2001).

2.1. Ground beef

Meat is defined as "the edible part of the skeletal muscle of an animal that was healthy at the time of slaughter". Ground beef is usually made from the less tender and less popular cuts of beef. Others more tender cuts can also be used (FSIS, 2001). The meat is composed of water (71 - 73 %), protein (20 - 22 %), lipid (5 - 8 %) and many other minor components (less than 1 %) like vitamins, enzymes, pigments and flavour compounds (Lambert *et al.*, 1991a). The proportion of these constituents gives the particular structure, texture, flavour, colour and nutritional value of the meat.

2.1.1. Consumption of ground beef

2.1.1.1.Canada

In Canada, the consumption of beef represent more than half of the total consumption of red meat (Statistique Canada, 2000). An average Canadian ate 31.0 kg of beef in 1999, an increase of 1.3 % from the year before.

2.1.1.2.United States of America

In the United States, the consumption of red meat in 1997 was evaluated at 50.3 kg per year, per person (USDA, 2001a). This represents a reduction of 1.6 % from the year before. The consumption of beef represents 57.5 % of the total consumption of red meat. An average American ate 28.9 kg of beef in 1997.

2.1.2. Economical aspect

2.1.2.1.Canada

In Canada, the beef industry is extremely important, with revenues close to 6.3 billions dollars in 1999, representing 20 % of all agricultural revenues (Agriculture et agroalimentaire Canada, 2001). From 1997, this represents an increase of 22.4 %. The exportation of beef increased by 7 %, reaching 421 935 tonnes in 1999. This represent 1.68 billions of dollars.

2.1.2.2.United States of America

In the United States, the beef industry is also important. The beef production was evaluated at 26.9 billions pounds in 2000, which represents an increase of 1 % from the previous year (USDA, 2001b).

2.1.3. Bacterial population

Ground beef is an excellent vehicle for bacteria. The two most important types of bacteria found in ground beef include pathogenic bacteria (such as *Salmonella, Escherichia coli* O157:H7, *Campylobacter jejuni, Listeria monocytogenes* and *Staphylococcus aureus*) and spoilage bacteria (such as *Pseudomonas* and *Lactobacillus*). Spoilage bacteria do not cause illnesses, but may cause deterioration of the ground beef, leading to bad odours.

2.1.3.1. *Escherichia coli* O157:H7

E. coli O157 : H7 is a facultative anaerobic gram-negative rod (Radomyski *et al.*, 1994). It is normally found in the gastrointestinal tract of warm blooded animals and humans (Mermelstein, 1993). There are about thousands of strains of *E. coli*, but only a small number is pathogenic to humans. These are called enteropathogenic *E. coli* and they can cause foodborne illness. The first outbreak of enteropathogenic *E. coli* was reported in 1982, after 47 people ate hamburgers contaminated with the bacteria.

The best known is *E. coli* O157:H7, which produces a cytotoxin called Shiga toxin 1 and/or Shiga toxin 2 (Buchanan and Doyle, 1997). The toxin responsible for severe and potentially fatal illness is called hemorrhagic colitis. The symptoms are bloody diarrhoea and severe abdominal pain. This bacteria can also cause a more rare disease, haemolytic uremic syndrome, the leading cause in kidney failure in children, thrombotic thrombocytopenic purpura in adults and damages to the central nervous system. The dose needed to become harmful is unknown. It is estimated that *E. coli* O157:H7 infects an estimated 73 000 persons a year in the United States (CDC, 2000a). In Canada, 1014 declared cases of foodborne illnesses was associated with *E. Coli* O157:H7 between January and October 2002 (Santé Canada, 2003).

E. coli O157:H7 is associated with dairy cattle and their product, milk and most of all, meat (beef, pork, poultry and lamb), meaning that food of animal origin is the main source of infection. The meat becomes contaminated with this bacteria in the slaughterhouse, but it is killed during normal cooking. With steaks, it is normally not a problem because the bacteria is present at the surface, and are easily killed during cooking. The problem is with the ground beef. During the process of grounding, the bacteria is distributed throughout the product, meaning *E. coli* O157:H7 is present everywhere. That is why it is important to cook the ground beef thoroughly.

2.1.3.2.*Salmonella*

All the serotype of *Salmonella* are potentially dangerous. The principal source of the infection is the consumption of contaminated food, like chicken, beef, milk, egg, crab, etc. (Radomyski *et al.*, 1994). The disease can be caused by eating a food product originally contaminated with the bacteria or by cross-contamination. *Salmonella* causes a gastro-intestinal infection, with severe symptoms in very young children and in elderly. The symptoms are fever, diarrhoea, in some cases, severe enough to cause dehydration, intestinal pain and vomiting. It can even cause death. The disease can last from a few days to a few weeks. A subsequent carrier state may persist in a asymptomatic person for up to several months. *S. typhi* may even be excreted intermittently throughout the person's life. In the United States, it is estimated the 1.4 millions persons get infected by *Salmonella* each year (CDC, 2000b). In Canada, 4816 declared cases of foodborne illnesses was associated with *Salmonella* between January and October 2002 (Santé Canada, 2003).

2.1.3.3.*Campylobacter jejuni*

C. jejuni is an important pathogen present in the intestinal track of several warm blooded animal like chicken and beef (Radomyski *et al.*, 1994). In Canada, the number of cases of campylobacteriosis is superior to the number of salmonellosis (Simard, 1995). In the mid nineties, the estimated cases were in the range of 1.1 millions to 7 millions in Canada (Welbourn, 1998). Between January and October 2002, 9264 cases of campylobacteriosis were declared (Santé Canada, 2003). The symptoms are, in general, mild. They vary from a low fever, cramps and nausea. The symptoms last between 2 to 10 days. Unfortunately, relapses can occur, leading to some complication. In the most extreme cases, it can cause meningitis, arthritis or the Guillain-Barre Syndrome (GBS), however, death is rare (Simard, 1995).

2.1.3.4.*Listeria monocytogenes*

Listeria monocytogenes was isolated in the faeces cattle, poultry, birds and fish. Most animals are healthy carriers (Bourgeois, *et al.*, 1996). That is why meat are often contaminated with the bacteria. *L. monocytogenes* will affect mostly new-born, elderly and immunocompromised people (Bourgeois, 1996; Adams and Moss, 1995). The infection will be associated with flu-like symptoms in mild cases or with a septicaemia associated or not with an infection of the central nervous system (meningitis, meningoencephalitis) in the most severe cases. Listeriosis has little affect on pregnant women, but can have severe consequences on the fœtus or can lead to a miscarriage. Mortality will affect 30 % of infected people. In Canada, listeriosis are extremely rare and in the United Stated, the number of cases is estimated at 2500 cases per year (CDC, 1999).

2.1.3.5.*Staphylococcus aureus*

Staphylococcus aureus is commonly present in food in small number in poultry especially in raw meat after contact with the microflora of the skin. The intoxication is caused by the ingestion of a toxin. The bacteria can produce seven types of protein exotoxins, A, B, C_1, C_2, C_3, D and E (Adams and Moss, 1995). The exotoxins most often associated with food poisoning are A and D. They are responsible for 77 % of the outbreaks. These toxins are heat stables. It is therefore possible to become ill after eating a food which contains no viable cells. Staphylococcal food poisoning is a relatively mild and short type of illness. Which would explained why it is more likely to be under-reported. Symptoms reported varies from nausea, vomiting and stomach cramps to diarrhoea, dehydration and fainting. In the United States, 185 000 cases are estimated each year (CDC, 1999).

2.1.3.6.*Pseudomonas* and *Lactobacillus*

Pseudomonas and *Lactobacillus* are bacteria responsible for meat spoilage (Bourgeois, *et al*, 1996). *Pseudomonas* will be responsible for the production of warnoff odours caused by superficial putrefaction and the development of a brown colour. *Lactobacillus* will be responsible for the greenish colour to the meat caused by production of sulfuric hydrogen and hydrogen peroxide which forms green pigments from the myoglobin.

2.1.4. Chemical composition

2.1.4.1.Lipids

The lipid content of ground beef is high. According to Canadian regulations, regular ground beef should not have a lipid content higher than 30 %, lean ground beef, 23 % and extra-lean, 17 % (Santé Canada, 2002). A typical composition of ground beef contains 18 % lipids and its fatty acids content is divided in 46 % saturated fatty acids, 51 % mono-unsaturated fatty acids and 3 % poly-saturated fatty acids (Johnson *et al*, 1994). The order of importance of the fatty acids composition normally found in ground beef is as followed : oleic acid (18:1), palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1), linoleic acid (18:2), linolenic acid (18:3) and arachidonic acid (20:4) (Giroux and Lacroix, 1998). The linoleic and arachidonic acid are important to the human diet, because they cannot be synthesised by the body.

2.1.4.2.Proteins

The globulin complex actomyosin is the main component of the muscle protein (Triebold and Aurand, 1963). It is composed of two proteins, actin and myosin. Actomyosin is responsible for the contractile properties of the muscles. It represents 38 % of the total muscle protein. Other proteins can also be found in smaller amount in the edible muscle tissues. First, collagen, reticulin and elastin which contribute to the structure and helps for the attachment to the skeleton. Myoglobin, on the other hand is the respiratory pigments responsible for the red colour of the meat. Finally, nucleoproteins are the main constituents of the genetic material and enzymes which are responsible for the metabolic reactions within the living cells.

2.1.4.3.Colour

The colour of the meat is very important to consumers. The red colour of ground beef is associated with good quality by consumers. Any other colour represent a product of poor quality. The red pigment of the meat is called myoglobin, a protein which possess ferric atom (Fe^{2+}) that resembles the hemoglobin in the blood of humans (Borgstrom, 1968). The myoglobin is mainly found in the centre of the meat, where oxygen is lacking. On the surface, the myoglobin is oxygenated and transformed in oxymyoglobin, giving a lighter red colour to the ground beef. The degree of formation of the oxymyoglobin depends on the amount of oxygen available. When the meat is refrigerated, there is more oxygen available than at room temperature for the myoglobin, because the oxygen consuming reactions on the surface of the meat, like bacterial activity, are suppressed at low temperature. In addition, the oxygen is more rapidly dissolved in the tissue of the meat at these temperature, in consequence, the refrigerated meat carries oxymyoglobin much deeper into the flesh than at room temperature. This pigment is unfortunately not stable if the exposure to oxygen is too long. It can be transformed in to metmyoglobin, which gives the brown colour to the meat. This discoloration is due to an oxidative reaction of Fe^{2+} to Fe^{3+}. If oxygen begins to disappear, the oxymyoglobin is transformed back into myoglobin and gives a darker red colour to the meat (Université Laval, 1997).

2.1.4.4.Taste

The flavour of the ground beef is influenced by lipids (de Roos, 1997). Lipids have an effect on our flavour perception, such as mouthfeel, taste and aroma, flavour stability and flavour generation. The proteins present in the ground beef will also influence the perception of flavour and taste (Fischer and Widder, 1997). They have an impact on the flavour release and the olfactory process. A small peptide naturally found in beef provides flavour enhancing properties. It is called the delicious peptide or the beefy-meaty peptide (BMP) or the savoury taste enhancing peptide (STEP).

2.1.5. Methods of conservation

Different methods of conservation of meat are used to prevent or slow down the process of deterioration caused by bacteria. The methods most commonly used are refrigeration, freezing, cooking, MAP (Université Laval, 1997). Refrigeration is the most used method for meat storage. The ideal temperature ranges from –2° C to 5°C. The use of refrigeration will slow the deterioration process before consumption. Refrigeration starts immediately after slaughter and continues through out the storage, transformation, transport and storage in the home. It is used to slow down bacterial growth and enzymatic and chemical activities responsible of meat deterioration. However, it is a short term solution, since the speed of deterioration increases with time. The shelf life of refrigerated meat depends on the initial bacterial count, the temperature and relative humidity during storage. After 3 to 5 days of storage in refrigerated condition, the meat is generally improper for consumption.

For a longer period of storage, freezing is an excellent way to preserve the quality of the meat (Université Laval, 1997). Freezing helps to preserve the colour, the flavour, the odour and the water content after cooking. The water crystal formed during freezing prevents bacterial multiplication. To prevent a major water loss, freezing has to be done quickly, the water crystal formed are smaller which reduces a water loss during defrosting. When frozen at –12°C, ground beef can be stored for up to 4 months.

Cooking the meat helps to destroy micro-organisms and inactivates enzymes responsible for deterioration (Université Laval, 1997). Cooking provokes the denaturation of proteins in the meat. This denaturation is responsible for the changes associated with cooking, like the colour, water retention and the texture of the meat. Cooking of fresh meat prolongs the shelf life in refrigerated condition by 1 to 2 weeks, depending on the length and the temperature of the cooking treatment (lower than 121°C). Under freezing condition, the shelf life can be between 14 to 15 months. Sterilisation of the meat (higher than 121°C) allows the conservation of the meat in a airtight container at room temperature for up to a year.

The use of modified atmosphere packaging (MAP) is well establish since the 1930s. It was used to transport fresh beef from Australia and New Zealand. MAP can potentially increase the shelf life by 50 % to 400 % (Farber, 1991). Spoilage bacteria can be destroyed by a reduction of the concentration of O_2 by increasing the concentration of CO_2. Optimal inhibition of deterioration bacteria can be reached with concentration of CO_2 of 40 % to 60 %. Under these conditions, the bacterial population shifts from gram-negative bacteria (deterioration bacteria) to a more predominant gram-positive facultatively anaerobic population (*Lactobacillus* ssp and *Brochothrix thermospacta*) (Farber, 1991; Lambert et al, 1991b).

Food irradiation is the newest treatment for prolonging the shelf life of ground beef. It will be discussed in detailed in the next section.

2.2. Food irradiation

The interest in irradiation began a long time ago. It started several decades ago with the irradiation of non-food polymers (car tires, wires insulation, printing inks, packaging films). It was also used to sterilise up to 50 % of medical disposable materials. Now irradiation is also used in cosmetics and for the sterilisation of some baby products. Then, the irradiation of food products to eliminate pathogens followed.

Food irradiation is now permitted in 37 countries (Thakur and Singh, 1994; Radomyski *et al*, 1994). Of those 37 countries, 26 are using irradiation on a commercial scale for one or more product (Giroux and Lacroix, 1998).

The exposition of food to ionising radiation is called food irradiation. It is a physical phenomenon of very high energy with great penetrating power (Thakur and Singh, 1994; Radomyski et al. 1994) and lethality due to the action at the cellular level (ICMSF, 1980). Ionising radiation has several advantages compared to other methods currently being used to destroy micro-organisms in food (ICMSF, 1980). The use of radiation is extremely lethal. The dose can however be adjusted for pasteurisation or sterilisation. When it is being used at very low dose (<5 kGy), there are no organoleptically detectable changes in the food. For ground beef, the dose is 3 kGy. And even with high doses (>10 kGy), the total chemical changes in the food are minimal. Finally, penetration of radiation is instantaneous, uniform and deep, allowing precise process control and does not cause any temperature increase.

There are also some disadvantages associated with food irradiation (ICMSF, 1980). With the doses used to treat food, the enzymes are not inactivated and remain active during storage. The chemical changes in the product due to the radiation, even though in small amount, may cause unacceptable organoleptic changes in sensitive foods such as salmon and green vegetables. In these two cases, the chemical changes will affect the appearance of the food product, making it less appealing to the consumer's eye.

Ionising radiation is applied to different materials, such as food to sterilise or prolonging the shelf life by destroying micro-organisms, parasites, and insects. Irradiation dose does not produce radioactive food because the energy produced during radiation is not enough to affect the neutrons in the nucleus (Doyle, 1999). The energy produced by the Co^{60} is not high enough to induce radioactivity (OMS, 1995). It is the process of removing electrons from atoms or molecules, changing them into ions. The nucleus of the atoms is not affected.

2.2.1. Sources of ionising radiation

2.2.1.1.Gamma rays

Gamma radiation is a high intensity radiation, with an excellent penetrating power produce by the decay of radioactive isotopes (ICMSF, 1980). Gamma rays can be produced by either cesium^{137} ($_{55}Cs^{137}$) or cobalt^{60} ($_{27}Co^{60}$) (Thakur and Singh, 1994). $_{55}Cs^{137}$ has a half life of 30 years. It emits γ–rays and β–particles to stabilise its self in $_{56}Ba^{137}$. To obtain $_{27}Co^{60}$, a neutron is added to $_{27}Co^{59}$ (a non-radioactive element). Once $_{27}Co^{60}$ is obtained, the element decays to form $_{28}Ni^{60}$ (a more stable element) and emits at the same time one β–particle and two γ-rays. This isotope is the one used most often. The produce isotope can penetrate food up to 40 cm thick (ICMSF, 1980). This type of radiation is the most commercially used, due to the high penetrating power, simple design and a uniform dose distribution (Thakur and Singh, 1994).

2.2.1.2.X-Rays

This type of radiation is produce when high-energy electrons bombard a piece of metal (Thakur and Singh, 1994). X-rays are produce usually under vacuum in a tube containing wire filament with a negative charge (cathode) and a piece of metal with a positive charge (anode) placed on opposite each other. When the filament is heated by an electric current, it discharges electrons that hit the piece of metal, producing x-rays. The potential difference between the cathode and the anode determines the energy and the wavelength of the radiation. Even with the high penetration power and dose rate, x-rays are not used in food irradiation because of the poor conversion of accelerated electrons to x-rays.

2.2.1.3.Electron Beam

The electron beam consists of a heated wire or electron gun in an evacuated tube as electron source (Thakur and Singh, 1994). The electrons are attracted by the positive end of the tube and are accelerated to a speed close to the speed of light. The potential difference between the cathode and the anode determines the speed of the electrons, higher the potential difference, higher is the speed. The penetrating power of the electron beam depends on its energy.

2.2.2. Mechanism of radiation

Atoms are excited when high energy hits them, causing the expulsion of electrons and the formation of ion pairs consisting of negatively charged electrons and the positively charged residues (Thakur and Singh, 1994). The effect of ionisation continues as ejected electrons hits other atoms, giving rise to a chain of reactions that ends when particle energies fall to a low level. Water is easily ionised and can be a primary source of ionisation in foods with secondary effects on other molecules. This would mean that it is more a result of water ionisation than of a direct hit.

When food containing water is irradiated, electrons are removed from the water molecules and chemical links are broken, resulting in free radicals. First, an electron is lost due to he effects of the gamma rays. Then ionised water molecules (H_2O^+) are formed. The ionised water molecules can be transformed in tohydroxyl ($OH\bullet$) radical and a hydrogen ion (H^+). Because of these free radicals, radiation sensitivity is higher in aqueous media than in dry form (Gürsel and Gürakan, 1997). The hydroxyl radical can interact with other components of the irradiated food, such as lipids and proteins.

2.2.2.1.Lipids

Irradiation of lipids leads to lipid oxidation. Fatty acid molecules (the main components of lipids) have an electron deficiency in the oxygen atom of their carbonyl group and at the point of unsaturated bonds. Due to the tendency of the oxygen to complete its valence shell of electrons, the reactions following cleavage caused by irradiation are directed toward the regions of oxygen atoms and at the double bonds. These reactions lead to the formation of free radicals. To be more stable, these radicals degrade and form CO_2, CO, H_2 hydrocarbons and aldehydes (Thakur and Singh, 1994).

When molecules of lipids are in presence of OH• and oxygen, a series of reactions follows. First, the OH• reacts with a hydrogen atom of a lipid to form a free lipid radical and water (initiation). This lipid free radical then reacts with oxygen to form a peroxide radical. The peroxide radical then reacts with another lipid molecules to form an hydroperoxide and another free lipid radical (propagation). The hydroperoxide is responsible for the rancid taste to food. This is a chain reaction and proceeds as long as there are free fatty acids (termination).

2.2.2.2.Proteins

The presence of oxygen blocks the reductive deamination of proteins by removing e^- and H• (Thakur and Singh, 1994). Sulfur containing and aromatic amino acids react more with free radicals than aliphatic amino acids. When amino acids containing sulfur moieties are irradiated, ammonia and sulfuric acid are produce (Lacroix and Ouattara, 2000). For example, OH• radicals react more with aromatic residues. With proteins, some groups that interact in isolated amino acids may not be exposed sufficiently to reactants such as free radicals. Because of their rigid spatial structure, the radicals forms after a radiation treatment, are held together in position and have a high chance of recombination with other radicals. Thus due to their configuration, proteins are more resistant to irradiation compared to isolated amino acids.

Proteins present in food are generally protected from radiation by other food components. The effects caused by radiation are negligible up to doses of 10 kGy. The radiation energy goes into denaturation of proteins rather than the destruction of constituent amino acids. The destruction is greater with heating than irradiation.

2.2.2.3.Effect of ionising irradiation on micro-organisms

Radiation causes damages to the DNA and RNA (ICMSF, 1980; Lacroix and Ouattara, 2000). These damages are caused by irreversible chemical modifications by rupture of the hydrogen bonds and the disulfide bridges. Since DNA and RNA are essential for the survival and the reproduction of the cell, irreversible damages will cause the death of the cell. The death of micro-organisms from exposure to ionising radiation is logarithmic. It has been shown that the use of low-dose irradiation can eliminate or greatly reduce the number of foodborne pathogens present in ground beef and extend the shelf life (Thayer, 1993). There are four main effect caused by radiation on DNA. First, lesions on the strands of DNA and ruptures between the strands can appear. Abnormal bridges between the bases can be created. Finally, another possibility is the hydration of a base, which would eliminate the complementary between two bases, blocking the reproduction of the cell.

The sensitivity of micro-organisms to ionising radiation varies from species to species (ICMSF, 1980; Murano, 1995; Lefebvre *et al.*, 1992). Gram-negative bacteria are more sensitive to radiation than gram-positive bacteria. This means that the most common food spoilage bacteria and enteric species (including pathogens : *Escherichia coli*, *Salmonella* spp, *Yersinia enterocolitica* and other members of the family Enterobacteriaceae) are sensitive to ionising radiation. There are, however, organisms that are resistant to radiation. The faecal streptococci and lactobacilli (Gram positive bacteria) are some what resistant, bacterial spore even more and *Micrococcus radiodurans* exceptionally resistant to irradiation.

Ionising radiation on meat can be used for two kinds of processing : radiation pasteurisation and radiation sterilisation. Radiation pasteurisation describes the use of low dose treatments of 2 to 5 kGy, which can leave numerous survivors. This type of radiation can be divided in two, depending on the objective. If the treatment is to extend the shelf life, it is called *radurization* and if the treatment is to eliminate one particular pathogen, it is called *radicidation*. The difference is arbitrary. It does not matter what the treatment is called, the effects are the same. Radurization eliminates pathogens and improves safety. Radiation sterilisation describes the use of higher doses for treatment and is used to produce a sterile product. Such an application is referred to by the term *radappertization* (ICMSF, 1980).

2.2.3. Factors affecting susceptibility of micro-organisms to irradiation

There are basically five factors that can affect the susceptibility of microorganism to irradiation (Murano, 1995). These are :

a) The dose of irradiation used. The curve representing the destruction of a bacteria by radiation represents a linear regression. This means that with a particular dose, the bacterial count will decrease by 1 log, then twice that dose will reduce the bacterial count by 2 log.

b) The temperature during irradiation treatment. By lowering the temperature, the formation of radicals will be slower, almost inhibited, due to the interaction of ionising energy with water molecules. The radicals affect indirectly the bacteria present in the product and interfere with their normal cellular functions. For example, it was demonstrated that the D_{10} of *Campylobacter jejuni* increased when the ground beef was under frozen condition (Lambert and Maxcy, 1984).

c) The headspace atmosphere within a package of food can affect the susceptibility of bacteria to irradiation. According to Murano (1995), there is no significant difference in the susceptibility of bacteria to irradiation when ground beef is under air or under vacuum packaging. However, Thayer and Boyd (1999) demonstrated that the irradiation treatment was significantly more lethal when the ground beef was packed under air than under either vacuum packed or under MAP containing a mixture of various concentrations of O_2, CO_2 and N_2. The presence of oxygen in air will increase the lethal effect of irradiation, due to the formation of oxygen radicals. Bacteria become more resistant to irradiation when packaged under N_2 (Murano, 1995).

d) The composition of the medium in which irradiation is applied. The D_{10} of bacteria depends on the media used. In general, bacteria is more resistant in food the in culture media. Also, the nutrients composition of the food product will have a protective effect on the bacteria. The susceptibility also varies with the food product (Murano, 1995).

e) The type of organism present in the food product. As a general rule, the simpler the life form, the more resistant it is to radiation. This is due to the richness of the DNA in the cell. The sensitivity will increase with the size and the degree of complexity of organisation in the cell. For example, viruses are more resistant than bacteria and bacteria are more resistant than humans. As mentioned earlier, the resistance also varies between bacteria, gram-positive being more resistant than gram-negative (ICMSF, 1980; Murano, 1995).

2.2.4. Effect of irradiation on organoleptic properties of food

It was shown that at the approved dose, there is no detrimental effect on the organoleptic quality of food (Murano, 1995). The limiting dose for pork is 1.75 kGy compared to 2.5 kGy for poultry and beef (Thakur and Singh, 1994). Above these doses, there is a chance of the development of off-odours, due to the oxidation of polyunsaturated fatty acids present in the phospholipids (Lacroix and Ouattara, 2000) and the oxidation of sulfur containing amino acids (Giroux and Lacroix, 1998). Experiments done with doses lower than 10 kGy demonstrated that the modification of the nutritional value of the product is not that different from those obtained with

more conventional methods (Giroux and Lacroix, 1998). In a study (Murano, 1995) on the sensory characteristics of ground beef irradiated at 2.0 kGy showed that the only difference after one day between irradiated and nonirradiated samples was that the irradiated sample were juicier and tender. After seven days, there was no significant difference between the irradiated and the nonirradiated samples.

It was shown, in an experiment done in water and some other solution, that the loss of vitamins could be significant (Doyle, 1999). However, like pathogenic bacteria, vitamins are less sensitive when in a complex matrix like food. It has to be mentioned that vitamins are also destroyed by cooking or other thermal preservation techniques. So the vitamin loss due to irradiation is not significantly different than other conventional processing.

2.2.5. Effect of irradiation on meat colour

It has been proven that the ionising radiation has an effect on the colour of the ground beef (Lacroix and Ouattara, 2000). After radiation treatment, the myoglobin or hemoglobin reacts with the free radicals like hydroxyl (OH•) and sulfuryl (SH•) radicals to form metmyoglobin and sulfmyoglobin (responsible for the green colour). By adding an antioxidant, the free radicals can react with that antioxidant and thus inhibit the formation of metmyoglobin and sulfmyoglobin, leaving the ground beef with its red colour. It was demonstrated by Giroux *et al.* (2001) that the addition of ascorbic acid to ground beef before irradiation helped to maintain the red colour of the meat after irradiation.

2.2.6. Regulations

Food and Agriculture Organisation (FAO), International Atomic Energy Agency (IAEA) and World Health Organisation (WHO) approved in 1981 the wholesomeness of food irradiation (Lacroix and Ouattara, 2000). They stated that a doses lower than 10 kGy had no effect on the nutritional aspect of a food product.

2.2.6.1.Canada

In Canada, food irradiation has been regulated for more than 30 years. At the beginning, irradiation was considered as an additive to food. After recommendation by Health and Welfare Canada to modify the reglementation of irradiated food (Agriculture Canada, 1987), Health Canada recognised food irradiation as a process (Food and Drugs Act). Four food products are approved for irradiation in Canada (MDS Nordion, 2001). The irradiation of potatoes was the first in 1960, followed by onions in 1965, to inhibit sprouting (0.15 kGy). In 1969, the irradiation of wheat and wheat flour was approved to control insects (0.75 kGy) and in 1984, vegetable seasonings (dried) and fresh herbs for decontamination (10 kGy). Five other food products are pending for approval. They are mangoes, shrimps, poultry, fruits and ground beef.

2.2.6.2.United States of America

The Food and Drugs Administration (FDA) and the Food Safety and Inspection Service (FSIS) both regulate food irradiation in the United States. Before 1986, only pork, fruits, spices, wheat flour, vegetable seasonings and potatoes had the seal of approval for irradiation in the United States of America (MDS Nordion, 2001). More recently, in 1992, the US Department of Agriculture (USDA) and Food Safety and Inspection Service (FSIS, 2001) permitted the irradiation of raw, packaged poultry (Radomyski *et al.* 1994; MDS Nordion, 2001) and in December of 1997, the irradiation of red meat was approved to control foodborne pathogens and other bacteria (Food and Drugs Administration, 1997; MDS Nordion, 2001). Presently, the irradiation of molluscan shellfish and crustacean shellfish are pending for approval (MDS Nordion, 2001).

2.2.7. Previous work on radurization and on radiosensitization

A study was done by Roberts and Weese (1998) to determine the shelf life of vacuum packed ground beef patties treated by gamma radiation, at different doses and stored at refrigerated temperature for 42 days. They found that the shelf life was dependant on the initial bacterial count. When the patties containing 10^4 CFU/g were irradiated at 1.0, 3.0, 5.0 and 7.0 kGy, the microbial shelf life was respectively 14, 21, 42 and > 42 days. Without irradiation, the ground beef patties had a shelf life of less than 7 days. Treatment at low dose of irradiation (< 3.0 kGy) probably eliminates the majority of the rapidly growing *Pseudomonas* and *Enterobacteriaceae* species, but allows radiation resistant bacteria, to grow. For example, lactobacilli species is predominant in irradiated ground beef (Ouattara *et al.*, 2002). The bacterial count of the lactobacilli is however reduced by irradiation at 5.0 and 7.0 kGy. In general, the product shelf life is determined by the ability of micro-organisms to repair some of damages caused to the DNA and the cell membranes.

Dempster *et al.*, (1985) demonstrated that when beef patties were irradiated at 1.03 and 1.54 kGy, an immediate bacterial reduction of 82 and 92 % was observed, and the shelf life was extended from 4 to 11 days by irradiation treatment. After 2 weeks of storage, the bacterial count was > 10^6 CFU/g when irradiated at 1.03 kGy and < 10^6 CFU/g when irradiated at 1.54 kGy. Irradiation also affected the colour at the surface of the patties but not the inside. The surface colour was more affected by the higher irradiation dose, being brighter. The colour intensity is related to the levels of myoglobin in the meat which is altered by irradiation. With storage, the colour of both the non-irradiated and irradiated samples became lighter, less red (Dempster *et al.*, 1985).

Radiosensitization was demonstrated by Lacroix *et al.* (1998) with a study on the irradiation of marinated chicken. The experiment consisted in comparing samples marinated in natural plant extracts (thyme, rosemary and lemon juice) before irradiation under air with samples irradiated under air and with irradiation under vacuum. The lowest bacterial count was found in the irradiated marinated samples. After an irradiation treatment of 3 kGy, the shelf life was 10, 11 and 15 days for samples irradiated under air, samples irradiated under vacuum and marinated samples combined with irradiation, respectively. Without irradiation, the shelf life was 2 days for samples under air and under vacuum and 7 days for marinated samples.

Farkas *et al.* (1992) demonstrated the synergistic effect of irradiation combined with a heat treatment on the spores of *Clostridium sporogenes* in canned luncheon meat. They demonstrated the a dose of 3 to 4 kGy had little effect on the spores of *C. sporogenes*, but when combined with a heat treatment of 105°C for 0.8 min this spoilage bacteria was controlled. With the heat treatment alone, 95 % of the spores remained viable. When combined with the irradiation treatment, 37 % remained. When soy hydrolysate (5 %) and lactic acid (5 %) was added, 32 % of the spores remained after the heat treatment and only 4 % remained in the canned luncheon meat.

Thayer *et al.* (1991) also examined the effects of heat (60°C for 3 min) and irradiation on *Salmonella typhimurium* in mechanically deboned chicken meat (MDCM). They demonstrated the heat the samples before irradiation had little effect in sensitizing the bacteria to irradiation, but when the heat treatment was done after the irradiation treatment, *Salmonella* was more sensible the heat treatment. Without irradiation, the concentration of *S. typhimurium* in the MDCM was 9.73 log CFU/g, and with heating, 5.79 log CFU/g. With irradiation treatment of 0.9 kGy, the concentration of *S. typhimurium* was 8.2 log CFU/g, in samples heated then irradiated, 3.38 log CFU/g and in samples irradiated then heated, 0.84 log CFU/g.

2.3. Additives

The use of additives to meat has been in practice since the prehistoric times. Meat and fish products were soaked in seawater to enhance the flavour and for its preservation. Today, a number of additives are used to prolong the shelf life of the meat product (Kabara, 1981). They can be found in spice extracts and in other natural or synthetic compounds.

2.3.1. Essential oils

The functional properties of the spices is found in the "essential fraction" (Conner, 1993). The antimicrobial activity of some essential oils, like thyme and rosemary have been known for several years. It was proven in 1989 that *E. coli* was more sensitive to thyme than rosemary (Farag *et al.* 1989). They can prolong the shelf life of foods by either a bacteriostatic or a bactericidal effect. Some extracts are also antioxidants, which prevents the rancidity of foods. The mechanism of bacterial inhibition by essential oils varies due the variety of antimicrobial compounds present. Unfortunately, the concentration needed to prevent bacterial growth is much higher than the concentrations currently being used in the industry for flavour (ICMSF, 1980). Another problem with the addition of essential oils, is that their is a loss of inhibitory activity after a certain period of incubation (Ouattara *et al.* 1997; Beuchat, 1976; Shelef *et al.* 1980; Conner, 1993).

2.3.1.1.Thyme and its constituents

Thyme essential oils is classified as a medium inhibitory antimicrobial additive (Conner, 1993). Beuchat (1976) reported the antibacterial effect of thyme extract on *Vibrio parahaemolyticus*. The essential oils of thyme at a concentration of 100 ppm prevented the growth of *V. parahaemolyticus* for about 5 hours. Juven *et al* (1994) also demonstrated that the addition of the essential oil of thyme to nutrient agar helped to decrease the viable count of *Salmonella typhimurium*. Thyme essential oils contains in majority thymol and carvacrol. These two volatile additives are responsible for the antimicrobial activity of thyme (Corner, 1993). They eliminate bacteria at a much lower concentration than thyme.

Thymol [5-methyl-2-(1-methylethyl)phenol] has been used as a therapeutic agent for years. It is now being considered as a antimicrobial agent. *Aspergillus parasiticus* in broth was eliminated for up to ten days in the presence of 500 µg/ml of thymol and for seven days with a concentration of 100 µg/ml (Buchanan and Shepherd, 1981). In another experiment, it was demonstrated that the addition of only 25 µg/ml of thymol to the media, inhibited the growth of *S. aureus* (Karapinar and Aktug, 1987).

Carvacrol is characterise as a compound with inhibitory effect on the growth of several pathogens (Ultee, *et al.*, 2000). It was demonstrated that at concentration of 0.15 % and higher, the growth of *Bacillus cereus* on rice was inhibited by at least 1 log (Ultee, *et al.*, 2000). Kim, *et al.* (1995a) also demonstrated a strong bactericidal effect of carvacrol on *E. coli* O157:H7, *Salmonella typhimurium, Listeria monocytogenes* and *Vibrio vulnificus*. On fish cubes, the addition of carvacrol at a concentration of 1.5 %, a good antibacterial activity was observed and at a concentration of 3 %, *S. typhimurium* was completely eliminated (Kim *et al.*, 1995b)

2.3.1.2.Rosemary and its constituents

Rosemary essential oils is classified as a medium inhibitory antimicrobial additives (Snyder, 1997). Rosemary is a known antioxidant, that can lower the thiobarbituric acid values in meat, but can also reduce the bacterial count (Shelef *et al.*, 1980). It was reported that the components of rosemary (rosmanol, rosmariquinone, rosmaridiphenol, carnosol) are up to four times more effective as an antioxidant than the antioxidant BHA and equal to BHT (Murphy, *et al.*, 1998). The addition of rosemary at a concentration of 0.3 % inhibited 46 bacteria in a growing media. Of these 46 bacteria, 21 were Gram positive, of which 9 were enteropathogenic (Shelef *et al.*, 1980). Farbood *et al.* (1976) demonstrated that rosemary extracts in growth medium at a concentration of 1.0 % had very little effect on *E. coli, E. aerogenes* and *P. fluorescens*, but a reduction of 43.2 % and 99.9 % of *S. typhimurium* and *S. aureus* was observed. When the effect of rosemary extracts was tested in meat, a bactericidal effect could be seen only on *S. aureus* at a much higher concentration, 5 %.

2.3.1.3.Trans-cinnamaldehyde

Trans-cinnamaldehyde is a major fraction of cinnamon, in a proportion of 75 % of oil (Bullerman *et al*, 1977). It was demonstrated that trans-cinnamaldehyde is the major component of cinnamon with the antimicrobial activity. It was shown that *E. coli* O157:H7, *S. typhimurium* and *Photobacterium leiognathi* were inhibited by the addition of trans-cinnamaldehyde at a concentration of 1 – 3 mM to the growing broth (Helander *et al.*, 1998).

2.3.2. Tannins

Plant tannins are water soluble phenolic compounds of vegetables (Chung, *et al.*, 1998). They are presents in several variety of fruits and vegetables, wines and teas. Tannins can act as natural antimicrobial compounds to protect fruits and vegetables against infections. It was also shown that tannins are potent antioxidants in various food and beverages (Khan and Hadi, 1998). The principal active tannin is tannic acid. Several bacteria, including *E. coli* and *S. typhimurium*, were inhibited by tannic acid (Chung, *et al.*, 1998).

2.3.3. Colour stabilisers

The colour is the first aspect of the meat that attracts the consumer. When the concentration of the metmyoglobin (responsible for the brown colour) reaches 30 to 40 % of the total pigments on the surface of the meat, the consumer will reject the meat. The discoloration can be caused by lipid peroxidation product and free radicals that will contribute to the oxidation of the oxymyoglobin to metmyoglobin.

The USDA has approved the addition of ascorbic acid to the surface of fresh beef or lamb cuts to stabilise the colour. Ascorbic acid is water-soluble and functions by contributing either one or two electron to more oxidised neighbouring species (Schaefer, *et al.*, 1995). Carnosine, another colour stabiliser, is a natural dipepdide (β-alanyl-L-histidine), that can be found in muscle. It can also reduce the lipid peroxidation in several food systems, and by doing so, stabilising the colour of the meat. The antioxidant activity of carnosine can be due to its ability to chelate transition metals such as copper, its enzyme-like activity or its free radical scavenging (Lee *et al.*, 1998).

It was also shown that ascorbic acid inhibited *Pseudomonas* sp. in broth (Costilow *et al.*, 1955). Later work by the same author demonstrated that the addition of ascorbic acid to meat had no measurable effect on the growth of bacteria on the surface. Lee *et al.* (1999a) demonstrated that the addition of ascorbic acid (0.1 %) to ground meat helped to reduce the formation of metmyoglobin on the surface and not in the bulk of the meat, where oxygen is more rare. On the other hand, they showed that the addition of carnosine (1.0 %) did significantly reduce the formation of metmyoglobin, not only on the surface, but all through the meat. Lee *et al.* (1998) demonstrated that the addition of carnosine to beef inhibited the formation of metmyoglobin during storage.

2.3.4. Bacteriocin

Bacteriocin is protein compounds produce by lactic bacteria, with a bacteriostatic or bactericidal effect on Gram positive bacteria (Nettles et Barefoot 1993). One bacteriocin that is being used since 1951 in the cheese production to prevent the formation of gas caused by *Clostridium*, is nisin, a GRAS (Generally Recognised as Safe) compound (Delves-Broughton 1990). It is produce by *Lactococcus lactis* subsp. *lactis* (Siragusa *et al.* 1999, Stevens *et al.* 1991 ; Delves-Broughton 1990 ; Harris *et al.* 1992) and has been approved for use in the production of cheese (Siragusa *et al.* 1999, Stevens *et al.* 1991), as a preservative in tomato juice, cream corn, beer and wine (Jung *et al.* 1992). It is also capable of preventing the growth of spores from *C. botulinum* (Jung *et al.* 1992, Stevens *et al.* 1991).

Since nisin affects the cytoplasmic membranes, only Gram positive bacteria are affected (Abbe *et al.* 1995 ; Nettles et Barefoot 1993, Stevens *et al.* 1991 ; Delves-Broughton 1990 ; Harris *et al.* 1992). Recent studies with beef carcass and the addition of nisin by spray (5000 Arbitrary Units/ml) showed that the bacterial population on the surface was reduced by 1.79 to 3.54 log UFC/cm (Cutter and Siragusa, 1994). Other studies done with artificially contaminated beef also showed that the addition of nisin delayed the bacterial growth of *L. monocytogenes* and *S. aureus* for one day, at room temperature (Chung, *et al.*, 1989). It was concluded that the use of nisin alone was not sufficient to control meat spoilage due to the presence of Gram negative bacteria.

Gram negative bacteria are not affected due to the presence of the outer membrane (Stevens *et al.* 1991). This problem can be solved by the addition of a chelator (Nettles et Barefoot 1993, Stevens *et al.* 1992). Experiments have been done with the addition of EDTA (ethylenediaminetetraacetate). Nettles and Barefoot (1993) demonstrated that *Salmonella, Shigella* and *E. coli* were affected by nisin in the presence of EDTA.

EDTA is a GRAS compound with several applications in the food and pharmaceutical industry as a chelator, antioxidant and as an antimicrobial agent (Saha *et al.* 1999). The addition of EDTA increases cellular permeability of Gram negative bacteria, by binding the magnesium ions in the lipopolysaccharide layer, thus increasing the susceptibility to antibiotics (Nettles et Barefoot 1993 ; Stevens *et al.* 1991 ; Stevens *et al.* 1992 ; Kabara 1981).

The addition of nisin (100 IU/ml) and EDTA (10 mM) completely inhibited *E. coli* in broth (Boziaris and Adams,1999). Stevens *et al.* (1992) demonstrated that the use of nisin at a concentration of 50 to 100 µg/ml and EDTA at 20 mM was effective against bacteria.

2.3.5. Antioxidants

Several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are currently being used in the industry to inhibit the formation of bad flavour. BHA and BHT are most commonly used antioxidant to prevent rancidity of food product containing lipids and also for their antimicrobial activity (Farag *et al.*, 1989). However, they are used less, due to the increase of concerns by consumers toward chemicals additives.

The addition of antioxidants to meat before cooking and irradiation provides a certain protection against lipids oxidation compared to irradiation alone without the addition of antioxidant (Lee *et al.*, 1999b). Antioxidants act as oxygen scavengers and reacts with free-radicals generated during cooking and irradiation. Lipid oxidation is responsible for the development of "warmed-off flavour". One way to prevent this development is with the addition of antioxidants to the meat (Chen *et al.*, 1984). The addition of antioxidants will also helps to prevent the oxidation of sulfur containing amino acids (Ouattara, *et al.*, 2002).

Some antioxidants are also effective against bacterial growth. It was shown that BHT was effective against *Salmonella* at a concentration varying between 400 and 10 000 ppm and BHA at a concentration of 150 ppm (Kabara, 1981). It was also cited that *A. flavus* was more sensitive to BHA than BHT. BHA is also more effective than BHT when tested against *Salmonella senftenberg* (Branen and Davidson, 1983)

2.3.6. Phosphate

Pyrophosphate compounds are the simplest of the condensed or polymeric phosphates (Ellinger, 1972). Pyrophosphates are composed of two phosphorus atoms linked through a shared oxygen atom. Tetrasodium pyrophosphate ($Na_4P_2O_7$) is a neutral crystalline sodium salt of phosphoric acid with sodium atoms in place of all the hydrogens. The addition of phosphate helps to increase moisture retention, and when combined with salt, the water-holding capacity of the meat tissues doubles. It was also demonstrated that the addition of pyrophosphate had an antimicrobial effect on some bacteria. Molins *et al.* (1984) demonstrated that tetrasodium pyrophosphate was highly inhibitory on pure cultures of *S. typhimurium, P. aeruginosa* and *S. aureus*. However, the addition of tetrasodium pyrophosphate to bratwurst had no effect on *S. aureus* (Molins *et al.*, 1985).

2.3.7. Other additives

Studies have demonstrated that the addition of various additives can inhibit the growth of both Gram positive and Gram negative foodborne pathogens. These compounds can be found in plant extracts, as organic acids group or phenolic compounds.

2.3.7.1.Plant extracts

The antimicrobial activities of plant extracts and essential oils used in different food product as flavouring agents have been recognised for several years. Oregano, classified as a medium inhibitory antimicrobial additives has an inhibitory effect against Mycotoxigenic *Aspergillus, Salmonella* ssp. and *Vibrio parahaemolyticus* (Snyder, 1997). Paster, *et al.* (1995) demonstrated that oregano exhibited fungicidal activity against *A. falvus, A. niger* and *Aspergillus ochraceus* on wheat grains. It was demonstrated by Beuchat (1976) that *V. parahaemolyticus* was slightly inhibited by nutmeg, curry powder, mustard and black pepper with a concentration range of up to 1 %.

The antimicrobial activities of essential oils are essentially due to some additives. For examples, eugenol from cloves, menthol from peppermint, vanillin from vanilla beans and anethole from aniseed and fennel essential oils are compounds present in essential oils with a wide spectrum of antimicrobial activity (Beuchat and Golden, 1989; Conner, 1993; Karapinar and Aktug, 1987).

2.3.7.2.Organic acids

Organic acids have been used for years for its antimicrobial activities. The activity of some organic acids can be as a fungicides or fungistats and others will be more effective in controlling the bacterial growth (Beuchat and Golden, 1989). The addition of organic acids will result in a reduction of pH of the substrate, a depression of the internal cellular pH by ionisation of the undissociated acid or disruption of substrate transport by alteration the permeability of cell membrane. Since the antimicrobial activity of organic acids is due to the undissociated portion of the acid molecule, the effectiveness depends on the dissociation constant (pK_a) of the acid. The pKa of most organic acids is between pH 3 and 5, this means that these acids are more effective at low pH values, i.e. the dissociated form.

Examples of organic acids with antimicrobial activities are citric acid, lactic acid and sorbic acid. Citric acid can be found in a variety of fruits, such as lemon, lime (Beuchat and Golden, 1989). It was demonstrated that the addition of 0.3 % of citric acid to poultry carcass was effective in reduce *Salmonella* (Thomson *et al.*, 1967). Citric acid was also shown to be effective in reducing the bacterial population in hard-cooked eggs (Fischer *et al.*, 1985) and in tomato juice (Murdoch, 1950).

Lactic acid is not normally found in food. It is the product of fermentation by lactic acid bacteria of food, such as sauerkraut, pickles, olives and some meats and cheeses (Beuchat and Golden, 1989). It was reported that lactic acid was able to inhibit spore forming bacteria at pH 5.0, but was unable to affect the growth of moulds and yeasts (Woolford, 1975).

Sorbic acid can be found in berries (Sofos and Busta (1981). Sorbic acid can be used for the preservation of human blood, animal feed, pharmaceutical, cosmetic products and packaging materials, but also as a food preservative of dairy products, bakery products, fruits and vegetable products and certain meat and fish product. The antibacterial effects of sorbic acid have been widely reported. It was shown by Zamora and Zaritzky (1987) that the addition of sorbic acid to beef was effective in reducing the total microbial population.

2.3.7.3.Phenolic compounds

Phenolic compounds are present in almost all plants (Beuchat and Golden, 1989; Caillet,1998). They are the result of secondary metabolism in plants. There is four group of phenolic compounds : 1. Benzoic acids and cinnamic acids; 2. Flavones and flavonols; 3. Chalcones, dihydrochalcones and aurones; 4. Anthocyanes (Caillet, 1998).

Phenolic compounds are known to contribute to the regulation mechanisms of fruit maturation and in defence reaction of plants (Caillet, 1998). They also possess an antimicrobial activity against some bacteria. It was determined by Powers *et al.* (1960) that anthocyanins had an inhibitory effect on the growth of *E. coli, S. aureus* and *Lactobacillus casei.* Phenolic compounds have also been suspected to be partly responsible for the antimicrobial activity of cranberries (Beuchat and Golden, 1989). It was shown that flavanols and proanthocyanidins in cranberries were highly inhibitory against *Saccharomyces boyancis*. The antimicrobial activity of flavanols was unaffected by pH. The activity of proanthocyanidins was enhanced at pH 5.2 and higher. Benzoic acid, which can be found in cranberries, raspberries, plums, prunes, cinnamon and cloves, is one of the oldest and most commonly used organic acid as food preservative. (Beuchat and Golden, 1989). The main function of this acid is as an antifungal agent in fruit-based and fruit-flavoured beverages, fruit products, bakery products and margarine.

Phenolic compounds also possesses antioxidant properties (Kalt, *et al.*, 2000; Rice-Evans. 2001; Zheng, W. and Wang, S.Y., 2001). The polyphenolic family is rich in flavonoids, an antioxidant phytochemicals (Rice-Evans, 2001). They are known to be powerful hydrogen–donating antioxidants and scavengers of reactive oxygen and reactive nitrogen. It was demonstrated by Kalt *et al.* (2000) that in blueberries, it is the anthocyanins that are responsible for the antioxidant activity.

2.4. Problematic

Ground beef is a very easily perishable food product and is associated with several food poisoning cases. Deterioration of the meat is also responsible for many recalls, resulting in several millions in loss. Currently used irradiation doses needed to help control the bacterial population in ground beef are high. At these doses, the taste, the flavour and the colour of the meat can be altered. That is why it is important to find other ways to reduce the irradiation dose used for treatment. The solution is to combine the irradiation treatments with the addition of additives with antimicrobial and/or antioxidant properties to obtain a synergistic effect. The addition of various additives would improve the efficacy of the irradiation treatment, without affecting the organoleptic qualities of the meat.

2.5. Hypothesis

The addition of various additives with antimicrobial and/or antioxidative properties to the ground beef prior to irradiation treatment would have a synergistic effect (combination of the killing effect of irradiation and of the selected additive) and would permit a reduction of the dose necessary to reduce or eliminate some pathogens present in ground beef. This would demonstrate the radiosensitization of *E. coli* and *S. typhi* in irradiated ground beef in presence of various additives.

2.6. Objectives

a) To determine the minimal inhibitory concentration (MIC) for various additives added to ground beef on *E. coli* and *S. typhi.*

b) To determine the irradiation sensitivity of *E. coli* and *S. typhi* in the presence of various additives with antimicrobial and/or antioxidant properties in ground beef.

c) To determine the best combination of additives with antimicrobial and/or antioxidant properties on the irradiation sensitivity of *E. coli* and *S. typhi* in ground beef.

d) To determine the effect of the packaging atmosphere on *E. coli* and *S. typhi* sensitivity during irradiation in ground beef in the presence of the best additives combination.

e) To determine the effect of irradiation temperature (4°C vs –80°C) on the sensitivity of *E. coli* and *S. typhi* in the presence of the best additives combination.

f) To determine the effect of selected additives, temperature and atmosphere conditions during irradiation on the stability of lipids.

2.7. Ways to reach the objectives

a) The minimal inhibitory concentration (MIC) will be determined by adding different concentrations of each additives to ground beef in the presence of *E. coli* or *S. typhi* (Bullerman *et al.*, 1977). Bacterial counts will be determined after an incubation period of ground beef samples for 24 hours at 4°C. Counts will be done on TSA at 35°C.

b) The irradiation sensitivity of *E. coli* and *S. typhi* will be determined by irradiating samples in the presence of various additives at various doses using the UC-15B irradiator (dose rate of 14.42 kGy/h) (Giroux *et al.*, 2001). *E. coli* and *S. typhi* will be plated at 35°C on TSA.

c) The best combination of additives will be determined by combining various additives in ground beef before irradiation (Giroux *et al.*, 2001). *E. coli* and *S. typhi* will be plated at 35°C on TSA.

d) The evaluation of the effect of headspace atmosphere conditions will be determined by irradiating ground beef samples under 100 % CO_2, under 60 %O_2 – 30 % CO_2 – 10 % N_2 and under vacuum in 0.5 mil metalized polyester / 2 mil EVA copolymer bag (205 mm x 355 mm, WINPACK, St-Léonard, Québec) (Giroux *et al.*, 2001). Results obtained with these gases will be compared with samples packed under air. *E. coli* and *S. typhi* will be plated at 35°C on TSA.

e) The evaluation of the effect of irradiation temperature will be determined at 4°C and –80°C (Giroux *et al.*, 2001). *E. coli* and *S. typhi* will be plated at 35°C on TSA.

f) The effect of irradiation on the stability of lipids will be evaluated by determining the thiobarbituric acid reactive substances, TBARS, content in ground beef samples containing various additives and packed under various atmospheres (Giroux, 2000). TBARS content will be determined by reporting optical density of samples on a standard curve.

3. Materials and Methods

3.1. Handling of the meat

Ground beef containing 23% of fat was purchased at a local supermarket (IGA, Laval, Canada) and transported to the Canadian Irradiation Centre (CIC) under refrigerated conditions (4 ± 2°C). The ground beef was vacuum-packed in portions of 450 g by the supermarket and immediately frozen at – 80°C in freezer. The frozen ground beef was sterilised at 25 kGy by irradiation using an underwater calibrator equipped with a ^{60}Cobalt source having a dose rate of 28.615 kGy/h (UC-15A; MDS Nordion, Kanata, ON, Canada). The irradiation treatments were carried out at the Canadian Irradiation Center (Laval, QC, Canada). The ground beef packs were stored at – 80°C until needed.

3.2. Preparation of bacterial cultures

Escherichia coli (ATCC 25922) and *Salmonella typhi* (ATCC 19430) were obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained at – 80°C in Tryptic Soy Broth (TSB; Difco Laboratory, Detroit, MI) with glycerol (10%; v/v). Before each experiment, stock cultures were standardised through two consecutive 24 h growth cycles in 10 ml of TSB at 35°C, without agitation to obtain working cultures containing approximately 10^9 CFU/ml for *E. coli* and *S. typhi*, in stationary phase.

3.3. Additives

Carnosine, carvacrol and trans-cinnamaldehyde were purchased from Aldrich (Milwaukee, WI, USA). Ascorbic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nisin, EDTA and tetrasodium pyrophosphate were purchased from Sigma (St Louis, MO, USA), tannic acid was purchased from ICN Biochemicals Inc (Aurora, OH, USA) and thymol was purchased from American Chemicals LTD (Montreal, QC, Canada). Essential oils from thyme (*Thymus satureioide*) and rosemary (*Rosmarinus officinalis cineoliferum* CT2) extract were obtained from Robert & Fils (Montreal, QC, Canada).

3.4. Determination of minimal inhibitory concentration (MIC) for each additive in ground beef

3.4.1. Preparation of the meat

The MIC of additives was determined on the basis of their antibacterial effectiveness in the meat matrix. Concentrations retained were those that reduce the population of *E. coli* or *S. typhi* in ground beef by 1 log CFU. Ground beef samples weighing 200 g were inoculated with working cultures of *E. coli* or *S. typhi* to obtain a final concentration of 10^5 CFU/g. The ground beef samples containing micro-organisms were mixed was 3 minutes at medium speed in a 4L-commercial blender (Waring Products, New Hartford, CO, USA) and different concentrations of the additives were incorporated, followed by another 3 minutes period of mixing. The ground beef samples containing micro-organisms and different concentrations of additives was distributed in sterile petri plates (60 x 15 mm)in portion of 25 g each and stored at 4°C for 24 hours.

3.4.2. Microbiological analysis

Samples of ground beef (10 g) were homogenised for 2 min in 90 ml of sterile peptone water (0.1%) using a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From the resulting homogenate, serial dilutions were prepared and appropriate dilutions were pour-plated in tryptic soy agar (TSA) (Difco, Laboratories, Detroit, MI, USA) and incubated at 35°C, 24 hours for the numeration of *E. coli* and *S. typhi.*

3.5. Concentration of the additives

The MIC values in the ground beef used for the determination of irradiation sensitivity are summarised in Table 1. The concentrations of some additives used in this study represent the minimum inhibitory concentrations (MIC) of the additives present in artificial culture media needed to reduce the growth by 1 log six pathogenic and spoilage bacteria, commonly found in

meat and meat products (unpublished data). Mean values of MIC were: 0.125 % for carvacrol; 0.5 % for rosemary; 0.2 % for thyme, 0.1 % for thymol; and 0.25 % for trans-cinnamaldehyde. The concentration used for carnosine (1.0 %; Sebranek, 1999), ascorbic acid (0.5 %; Giroux 2000) and tannic acid (0.38 %) was selected from the literature. The concentrations of BHA (0.01 %), BHT (0.01 %), EDTA (100 ppm), and tetrasodium pyrophosphate (0.1 %) corresponded to the concentrations recommended by the Canadian Food Inspection Agency (CFIA).

The concentration of the additives used in this study are summarised in Table 1

Table 1 : List of additives and their concentrations

Additives	Concentrations	
	E. coli	*S. typhi*
Ascorbic acid (w/w)	0.5 %[5]	0.5 %[5]
BHA (w/w)	0.01 %[4]	0.01 %[4]
BHT (w/w)	0.01 %[4]	0.01 %[4]
Carnosine (w/w)	1.0 %[3]	1.0 %[3]
Carvacrol (v/w)	0.125 %[1] and 0.88 %[2]	0.125 %[1] and 1.15 %[2]
EDTA (w/w)	100 ppm[4]	100 ppm[4]
Nisin (v/w)	625 UI/g[2]	625 UI/g[2]
Rosemary (v/w)	0.5 %[1]	0.5 %[1]
Tannic acid (w/w)	0.38 %[6]	0.38 %[6]
Tetrasodium pyrophosphate (w/w)	0,1 %[4]	0,1 %[4]
Thyme (v/w)	0.2 %[1] and 2.33 %[2]	0.2 %[1] and 2.75 %[2]
Thymol (v/w)	0.1 %[1] and 1.15 %[2]	0.1 %[1] and 1.6 %[2]
Trans-cinnamaldehyde (v/w)	0.025 %[1] and 1.5 %[2]	0.025 %[1] and 0.89 %[2]

[1]MIC values in artificial culture media against six pathogenic and spoilage bacteria.
[2]MIC values in ground beef.
[3]Selected from literature (Sebranek, 1999).
[4]Recommended by the Canadian food Inspection Agency (CFIA)
[5]Selected from literature (Giroux, 2000)
[6]Selected from literature

3.6. Preparation of the meat and inoculation procedures for D_{10} and lipid oxidation determination

3.6.1. D_{10} determination of each additives

Ground beef samples weighing 450 g were inoculated with working cultures of *E. coli* or *S. typhi* to obtain a final concentration of 10^5 CFU/g. The ground beef samples containing micro-organisms was mixed for 3 min at medium speed in a 4L-commercial blender (Waring Products, New Hartford, CO, USA) and the appropriate concentration of each additive were incorporated, by another 3 min of mixing. Ground beef samples in portion of 25 g containing micro-organisms and additives were filled in sterile petri plates (60 x 15 mm) each and stored at 4°C until irradiation treatment (approximately 15 h).

3.6.2. Determination of the effect of various concentrations of carvacrol on irradiation sensitivity of *E. coli* and *S. typhi*

The effect of various concentrations of carvacrol in irradiated ground beef was evaluated to determine if a smaller concentration of carvacrol would have the same effect on the irradiation sensitivity of *E. coli* and *S. typhi*. Sterile ground beef was inoculated with either *E. coli* or *S. typhi* to obtain a final concentration of 10^5 CFU/g. Various concentrations of carvacrol ranging from 0 to 2.0 % were added to the inoculated ground beef samples. Ground beef samples containing micro-organisms and additives was filled in sterile petri plates (60 x 15 mm) in portion of 25 g each and stored at 4°C until irradiation treatment (approximately 15 h).

3.6.3. Determination of the best combination of additives on irradiation sensitivity of *E. coli* and *S. typhi*

With the results obtained for the D_{10} values of each additives (results of the previous section), the additives selected to determine the best combination are carvacrol (1.0 %), ascorbic acid (0.5 %) and tetrasodium pyrophosphate (0.1 %). Carvacrol was chosen for its ability to increase irradiation sensitivity of *E. coli* and *S. typhi*. Ascorbic acid was chosen for its ability to maintain the colour of the ground beef during irradiation treatment. Finally, tetrasodium pyrophosphate was chosen for its ability to maintain the taste of the ground beef during irradiation treatment. The combination tested were carvacrol, a mixture of carvacrol and ascorbic acid, a mixture of carvacrol and tetrasodium pyrophosphate and a mixture of carvacrol, ascorbic acid and tetrasodium pyrophosphate.

Samples of ground beef were prepared with the different combination of additives as described in section 3.6.1.

3.6.4. Determination of the irradiation sensitivity of *E. coli* and *S. typhi* under various atmospheric conditions in the presence of the best combination of additives

The best combination (a mixture of carvacrol and tetrasodium pyrophosphate) was used to determination the irradiation sensitivity of *E. coli* and *S. typhi* under various atmospheric conditions. Samples of ground beef were prepared with the different combination of additives as described in section 3.6.1. One modification was made in the packaging of the meat. Ground beef samples containing micro-organisms and additives were packed in portion of 25 g each in 0.5 mil metalized polyester / 2 mil EVA copolymer bag (205 mm x 355 mm, WINPACK, St-Léonard, Québec). The bags were sealed under vacuum, under air : 78.1 % N_2 – 20.9 % O_2 – 0.036 % CO_2, under 100 % CO_2 or under modified atmosphere packaging (MAP) condition : 60 % O_2 - 30% CO_2 - 10% N_2 using a Sipromac Vacuum Packaging Machine. The bags were stored at 4°C until irradiation treatment (approximately 15 h).

3.6.5. Determination of the irradiation sensitivity of *E. coli* and *S. typhi* under frozen condition in the presence of the best combination of additives

The best combination (a mixture of carvacrol and tetrasodium pyrophosphate) was used to determination the irradiation sensitivity of *E. coli* and *S. typhi* under frozen condition. Irradiation treatment was done under the temperature used for pasteurisation (4°C) and for sterilisation (-80°C). Samples of ground beef were prepared with the different combination of additives as described in section 3.6.1., except samples were stored at 4°C or at –80°C until irradiation treatment (approximately 15 h).

3.6.6. Determination of lipid oxidation

Non sterile ground beef was mixed under air conditions with carvacrol (1.0 %), ascorbic acid (0.5 %), tetrasodium pyrophosphate (0.1 %), a mixture of carvacrol (1.0 %) and ascorbic acid (0.5 %), a mixture of carvacrol (1.0 %) and tetrasodium pyrophosphate (0.1 %) or with a mixture of carvacrol (1.0 %), ascorbic acid (0.5 %) and tetrasodium pyrophosphate (0.1 %). The best combination in term of D_{10} values (carvacrol (1.0 %) and tetrasodium pyrophosphate (0.1 %)) was also evaluated for thiobarbituric acid reactive substances (TBARS) content under various atmosphere (air (78.1 % N_2 – 20.9 % O_2 – 0.036 % CO_2); 100 % CO_2; MAP (60 % O_2 – 30 % CO_2 – 10 % N_2) and under vacuum) at 4°C and under frozen under air atmosphere (- 80°C). For each atmospheric conditions, samples without additives were analysed as a control. For each treatment, three samples (25 g) of ground beef were placed in small petri dishes for samples under air. Samples treated under MAP, CO_2 and vacuum were placed in a 0.5 mil metalized polyester/2 mil EVA copolymer bag (205 mm x 355 mm, WINPACK, St-Léonard, Québec). Two doses of irradiation were evaluated (0 kGy and 1 kGy).

3.7. Irradiation

The Irradiation treatments of ground beef samples for D_{10} determination and lipid oxidation determination were done using UC-15B irradiator (MDS-Nordion International Inc., Kanata, ON, Canada) equipped with a ^{60}Co source at a dose rate of 14.42 kGy/h. Irradiation doses used for D_{10} determinations ranged from 0.1 to 0.6 kGy for *E. coli* and from 0.50 to 2.0 kGy for *S. typhi*. Under frozen condition, the irradiation doses ranged from 0.1 to 0.7 kGy for *E. coli* and from 0.5 to 3.0 kGy for *S. typhi*. For the determination of the effect of various concentrations of carvacrol on the irradiation sensitivity, samples were irradiated at 0.25 kGy for *E. coli* and at 0.5 kGy for *S. typhi*. Samples were immediately analysed after irradiation to determine the microbial count. Irradiation dose used for the determination of lipid oxidation was 1 kGy.

3.8. Microbiological analysis

Samples of ground beef (10 g) were homogenised for 2 min in 90 ml of sterile peptone water (0.1%) using a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From the resulting homogenate, serial dilutions were prepared and appropriate dilutions were pour-plated in tryptic soy agar (TSA) (Difco, Laboratories, Detroit, MI, USA) and incubated at 35°C for 24 hours for the numeration of *E. coli* and *S. typhi*.

3.8.1. Normalisation of curves and calculation of D_{10} values

Normalisation of each curves was done to have a better idea of the combined effect of each additive with irradiation compared to the control. Normalisation will help to obtain the same Y – axis for all the additives and the control. Normalisation was obtained with the following equation:

$$\text{Multiplication factor} = \frac{\text{average of each values (log CFU / g) obtained for the control at 0 kGy}}{\text{Each values (log CFU / g) obtained for each additive at 0 kGy}}$$

The average of the multiplication factors is multiplied by each value (log CFU / g) at all the different doses used to obtain normalised values. The average of the normalised values for each irradiation doses is calculated. The curves for each additives are established with the average of the normalised values. The D10 value for irradiation in the presence of each additive corresponds to

$$D_{10} = -1 / m$$

D_{10} : Irradiation sensitivity

m : Slope of the curve

The efficiency of each additive in combination with the irradiation treatment was calculated to show the effect of each additive in comparison with the others additives used. It was calculated with the following equation :

$$\text{Efficiency} = \frac{D_{10} \text{ value of the respective control} - D_{10} \text{ value in the presence of the additive}}{D_{10} \text{ value of the respective control}} \times 100$$

3.9. Determination of lipid oxidation

Lipid oxidation was evaluated by determining the TBARS (μM/g) content in the ground beef based on a method described by Giroux (2000). TBARS evaluation was done at day 1 of storage, just after irradiation treatment. First, 10 g of ground beef was mixed with 50 ml of H_2O, treated by inverse osmosise, for 2 minutes in a Stomacher (Lab Blender 400, Seward Medical UAC House, London, England. The mixture was combined with 10 ml TCA (10 %), centrifuged for 10 minutes (3200 g) and filter through a Whatman #1. The filtrate (8 ml) was incubated with 2 ml thiobarbituric acid (TBA - 0.67 %) in a water bath (80°C) for 90 minutes.

TBARS was determined by measuring optical density of the samples at 532 nm and reporting on a standard curve. The standard curve used was as described by Lawlor *et al.* (2000). The technique consisted in determining the optical density (532 nm) of various concentrations (0 to 10 μM) of 1,1,3,3 – tetraethoxypropane (TEP) with thiobarbituric acid (TBA). It is important to note that the percentage of recuperation of TBARS is 89.8 %, as determined by Lawlor *et al.* (2000). This percentage was taken into consideration when the standard curve was established.

3.10. Statistical analyses

3.10.1. Experimental design

D_{10} determination of each additives : For microbial analysis, each bacterial analysis (*E. coli* and *S. typhi*) was done in two replicates and for each replicate, three samples of ground beef (25 g) were analysed in duplicates. Six concentrations of each additive were done for each analysis.

Determination of irradiation sensitivity (D_{10}) in the presence of each additive : For microbial analysis (*E. coli* and *S. typhi*), each bacterial analysis was done in two replicates and for each replicate, three samples of ground beef (25 g) were analysed in duplicates. Nineteen additives were evaluated for each analysis at six or seven irradiation doses.

Irradiation sensitivity (D_{10}) in the presence of additive combinations: For microbial analysis (*E. coli* and *S. typhi*), each bacterial analysis was done in two replicates and for each replicate, three samples of ground beef (25 g) were analysed in duplicates. Five combinations of additives were evaluated for each analysis at six or seven irradiation doses.

Irradiation sensitivity of microorganisms in the presence of the mixture of carvacrol and tetrasodium pyrophosphate under modified atmosphere : For microbial analysis (*E. coli* and *S. typhi*), each bacterial analysis was done in two replicates and for each replicate, three samples of ground beef (25 g) were analysed in duplicates. Four different atmospheres were evaluated for each analysis at six or seven irradiation doses.

Irradiation sensitivity in the presence of the mixture of carvacrol and tetrasodium pyrophosphate under two temperatures : For microbial analysis (*E. coli* and *S. typhi*), each bacterial analysis was done in two replicates and for each replicate, three samples of ground beef (25 g) were analysed in duplicates. Two irradiation temperatures were evaluated for each analysis at six or seven irradiation doses.

TBARS determination : For TBARS analysis, the experiment was done in two replicates and for each replicates, three samples were analysed. In the first experiment, seven additives under two irradiation doses were evaluated. For the second experiment, the combination of two additives under four atmospheres and treated with two irradiation doses were done.

3.10.2. Microbiology : D_{10} determination

The kinetics of bacteria destruction by irradiation with or without the additives and under different packaging conditions was evaluated by linear regression. Bacterial counts (log CFU/ml) were plotted against irradiation doses or compounds concentrations and the D_{10} values were calculated using SigmaPlot program. Statistical analysis were done by using SPSS program. Comparison of means were based on Duncan's multiple range test (probability of 0.05) and Student's t-test for paired samples between controls and treated samples (probability of 0.05).

3.10.3. TBARS determination

Comparison of means were based on Duncan's multiple range test (probability of 0.05) and Student's t-test for paired samples between controls and treated samples (probability of 0.05).

4. Results

4.1. Determination of MIC for each additives in ground beef

Table 2 and figures 1 and 2 shows the relative sensitivity of *E. coli* and *S. typhi* to the different additives under study. Results of MIC values are expressed in term of D_{10} (%) of additives concentration needed to reduce the total bacterial population by 1 log. The additives with the highest inhibitory effect on *E. coli* were carvacrol, thymol, trans-cinnamaldehyde and thyme, with MIC values of 0.88 %, 1.14 %, 1.57 % and 2.33 % respectively. These additives were followed by ascorbic acid, with a concentration of 2.71 %. The inhibitory effect of ascorbic acid was not significantly different ($p > 0.05$) compared to meat containing trans-cinnamaldehyde or thyme. The addition of rosemary or tannic acid had the least inhibitory effect on *E. coli*, with MIC values of 10.37 % and 11.15 % respectively.

Results obtained with *S. typhi* were slightly different then those obtained with *E. coli*. With *S. typhi*, the additives with the highest inhibitory effect were trans-cinnamaldehyde, carvacrol, thymol and ascorbic acid, with MIC values of 0.89 %, 1.15 %, 1.60 % and 1.83 % respectively. Thyme followed with a MIC value of 2.75 %. No significant difference ($p > 0.05$) was observed between thymol, ascorbic acid and thyme. The addition of tannic acid and rosemary had the least inhibitory effect on *S. typhi*. For those additives, the MIC values were 13.56 % and 21.18 %.

With these results, only the MIC values in ground beef of carvacrol, thyme, thymol and trans-cinnamaldehyde were used for the determination of the irradiation sensitivity of *E. coli* and *S. typhi* in ground beef.

Table 2: The minimal concentration of each additive needed to reduce the bacterial population of *E. coli* and *S. typhi* by 1 log in ground beef

Additives	MIC (%)[3]	
	E. coli	*S. typhi*
Carvacrol [2]	0.88 ± 0.12^{a}	1.15 ± 0.02^{a}
Thymol [1]	1.14 ± 0.05^{a}	1.60 ± 0.00^{ab}
Trans-cinnamaldehyde [2]	1.57 ± 0.10^{b}	0.89 ± 0.03^{a}
Thyme [2]	2.33 ± 0.32^{ab}	2.75 ± 0.17^{b}
Ascorbic acid [1]	2.71 ± 0.26^{b}	1.83 ± 0.06^{ab}
Rosemary [2]	10.37 ± 1.14^{c}	13.56 ± 1.28^{c}
Tannic acid [1]	11.15 ± 2.04^{c}	21.18 ± 2.07^{d}

[1] Percentage (w/w)

[2] Percentage (v/w)

[3] Duncan – [a, b, c, d] Values in same columns with different letters are significantly different ($p \leq 0.05$)

Tableau 2a: Concentration minimale de chacun des additifs nécessaire pour réduire la population bactérienne d'*E. coli* et *S. typhi* d'un log dans du bœuf haché

Additifs	MIC (%)[3]	
	E. coli	*S. typhi*
Carvacrol [2]	$0,88 \pm 0,12^{a}$	$1,15 \pm 0,02^{a}$
Thymol [1]	$1,14 \pm 0,05^{a}$	$1,60 \pm 0,00^{ab}$
Trans-cinnamaldéhyde [2]	$1,57 \pm 0,10^{b}$	$0,89 \pm 0,03^{a}$
Thym [2]	$2,33 \pm 0,32^{ab}$	$2,75 \pm 0,17^{b}$
Acide ascorbique [1]	$2,71 \pm 0,26^{b}$	$1,83 \pm 0,06^{ab}$
Romarin [2]	$10,37 \pm 1,14^{c}$	$13,56 \pm 1,28^{c}$
Acide tannique [1]	$11,15 \pm 2,04^{c}$	$21,18 \pm 2,07^{d}$

[a] Pourcentage (p/p)

[b] Pourcentage (v/p)

[3] Duncan [a, b, c, d] Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0.05$)

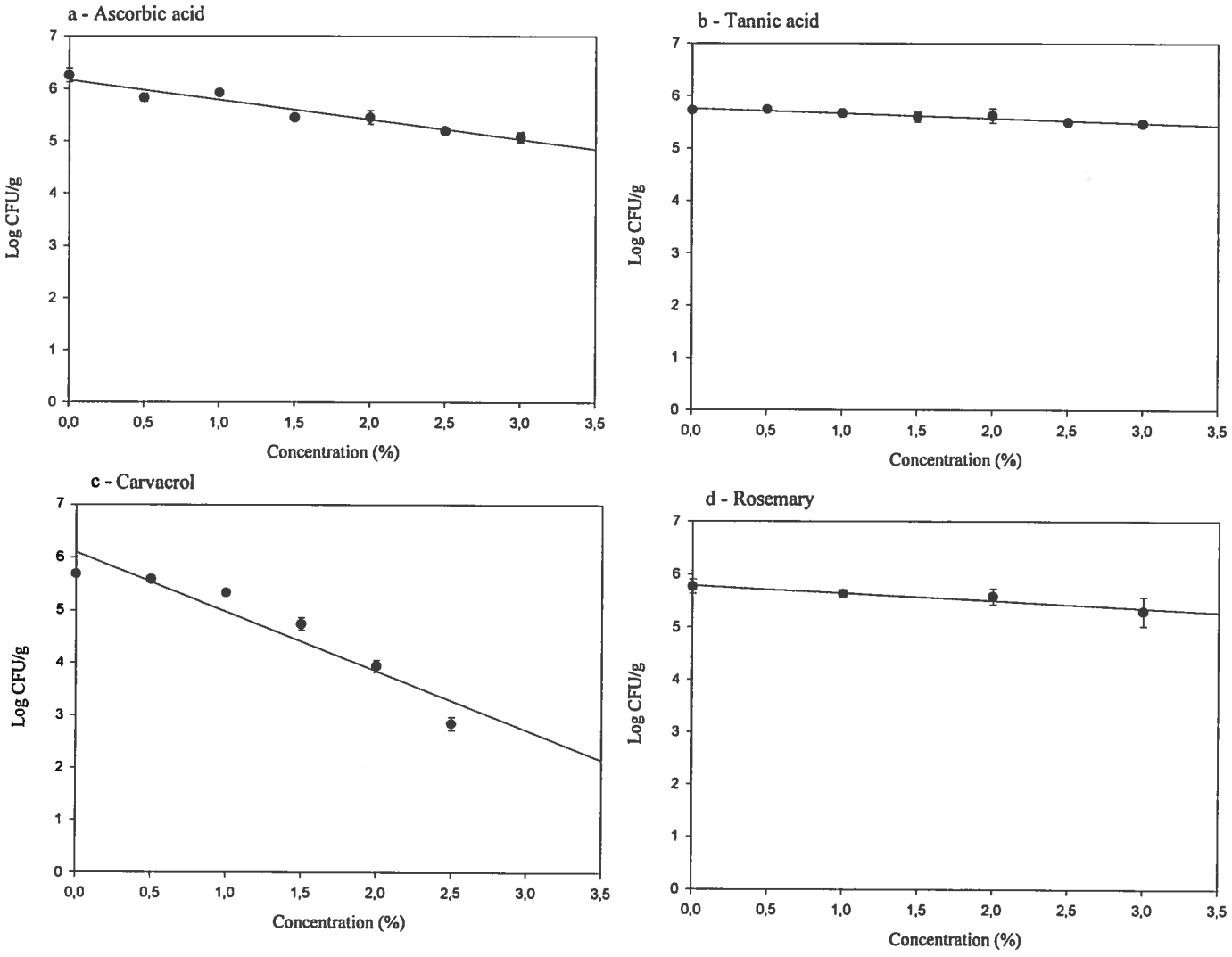


Figure 1 : Antimicrobial effectiveness of different additives on *E. coli* in ground beef

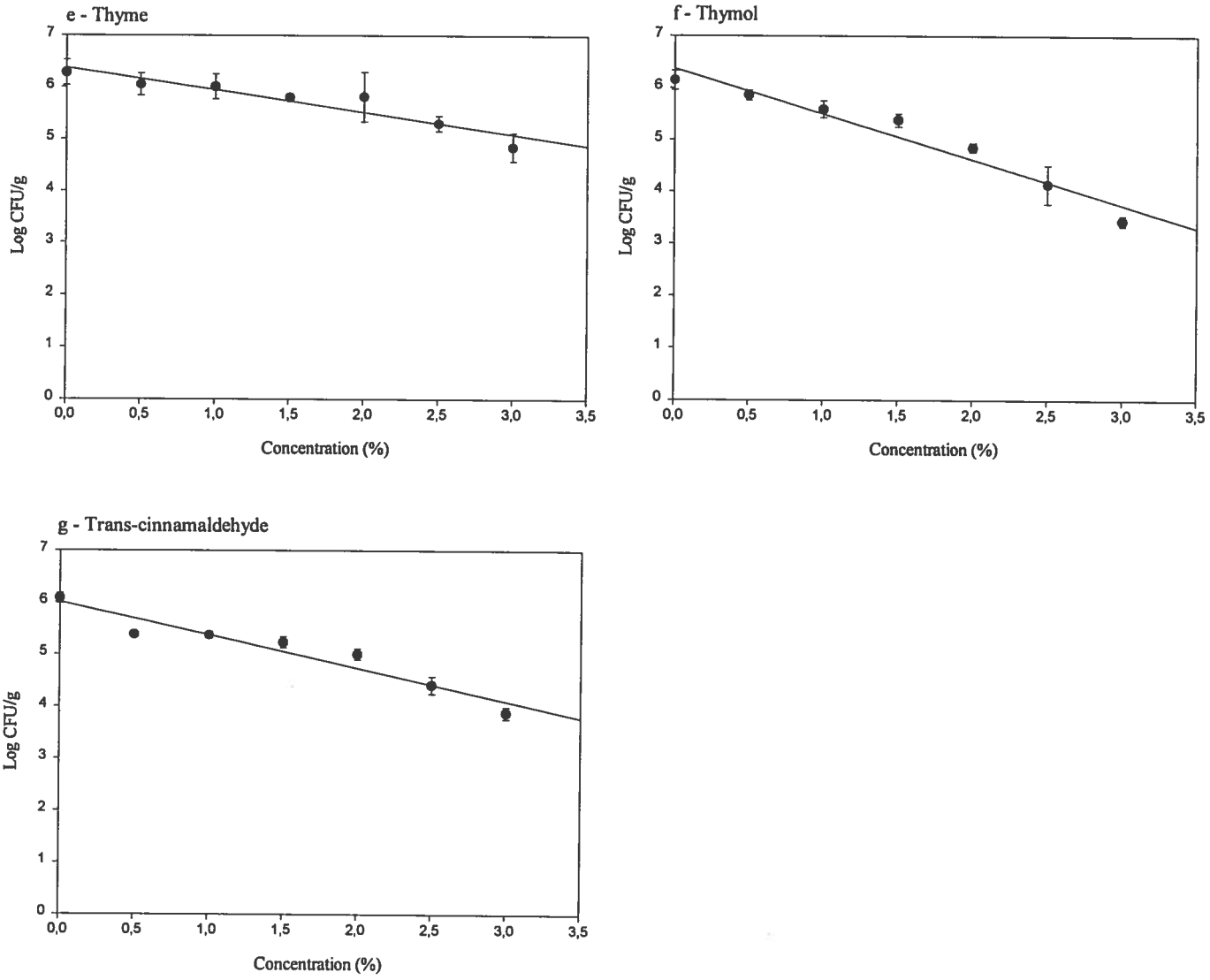


Figure 1 (contd.) : Antimicrobial effectiveness of different additives on *E. coli* in ground beef

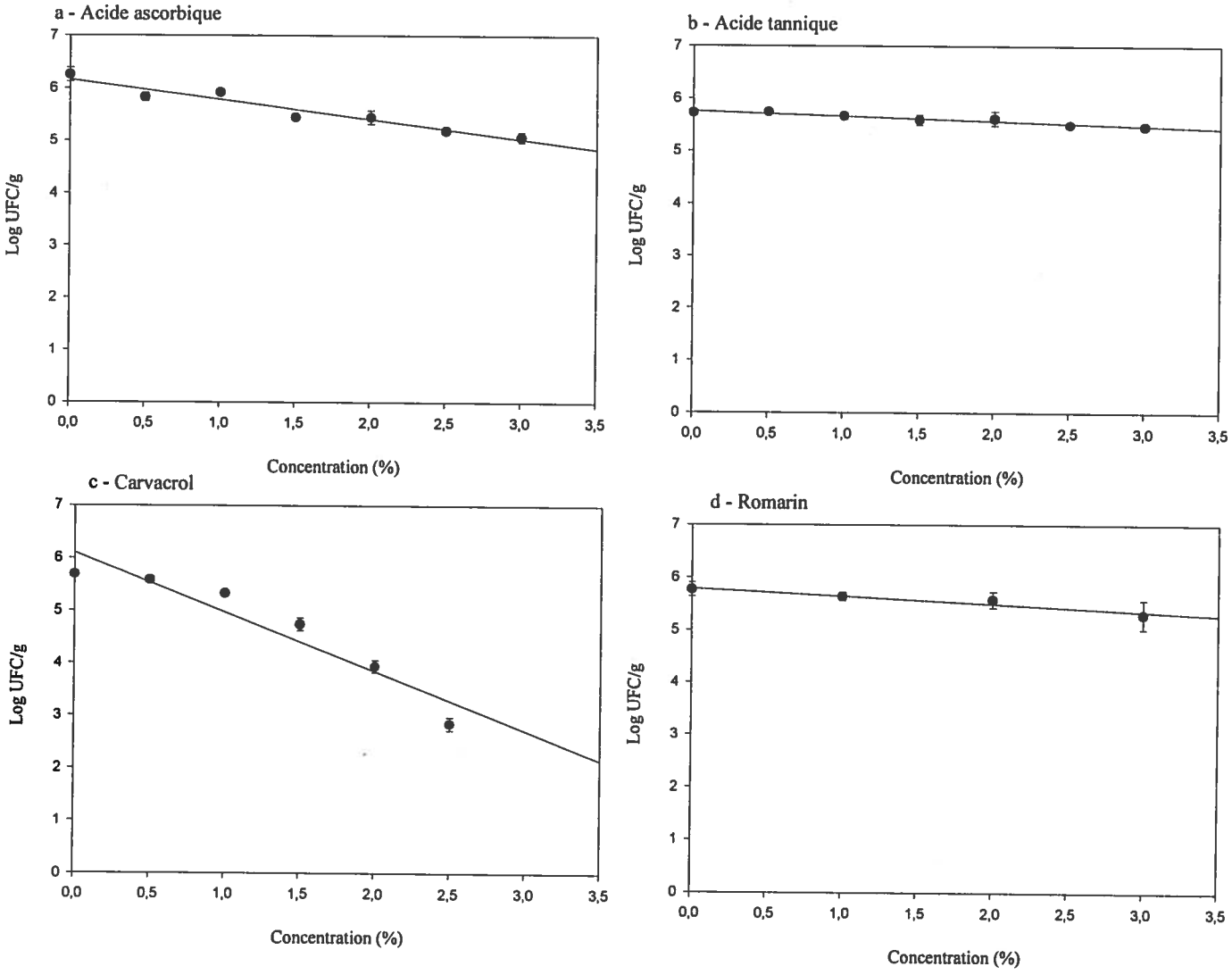


Figure 1a : Efficacité antimicrobienne de différents additifs sur *E. coli* dans du bœuf haché

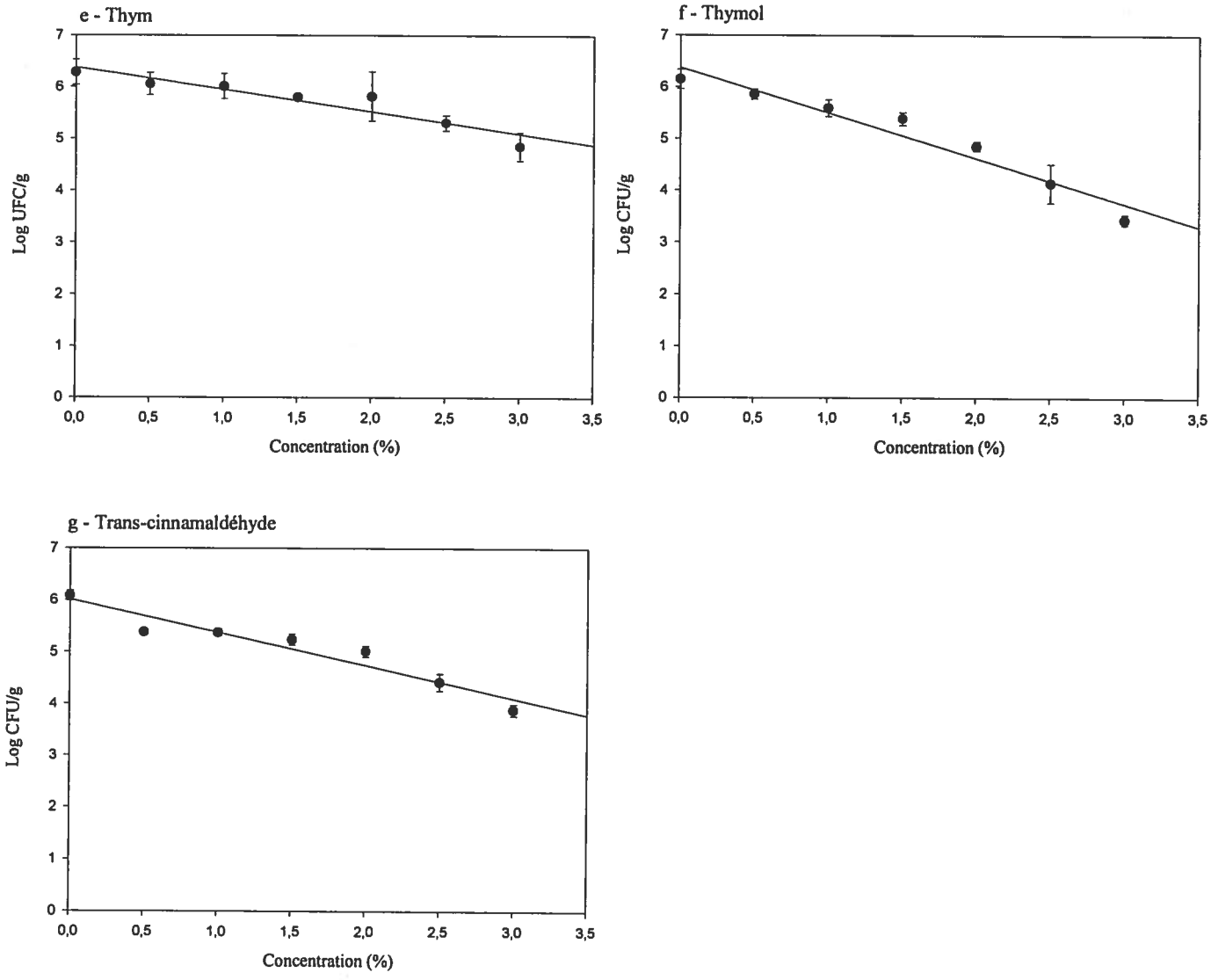


Figure 1a (suite) : Efficacité antimicrobienne de différents additifs sur *E. coli* dans du bœuf haché

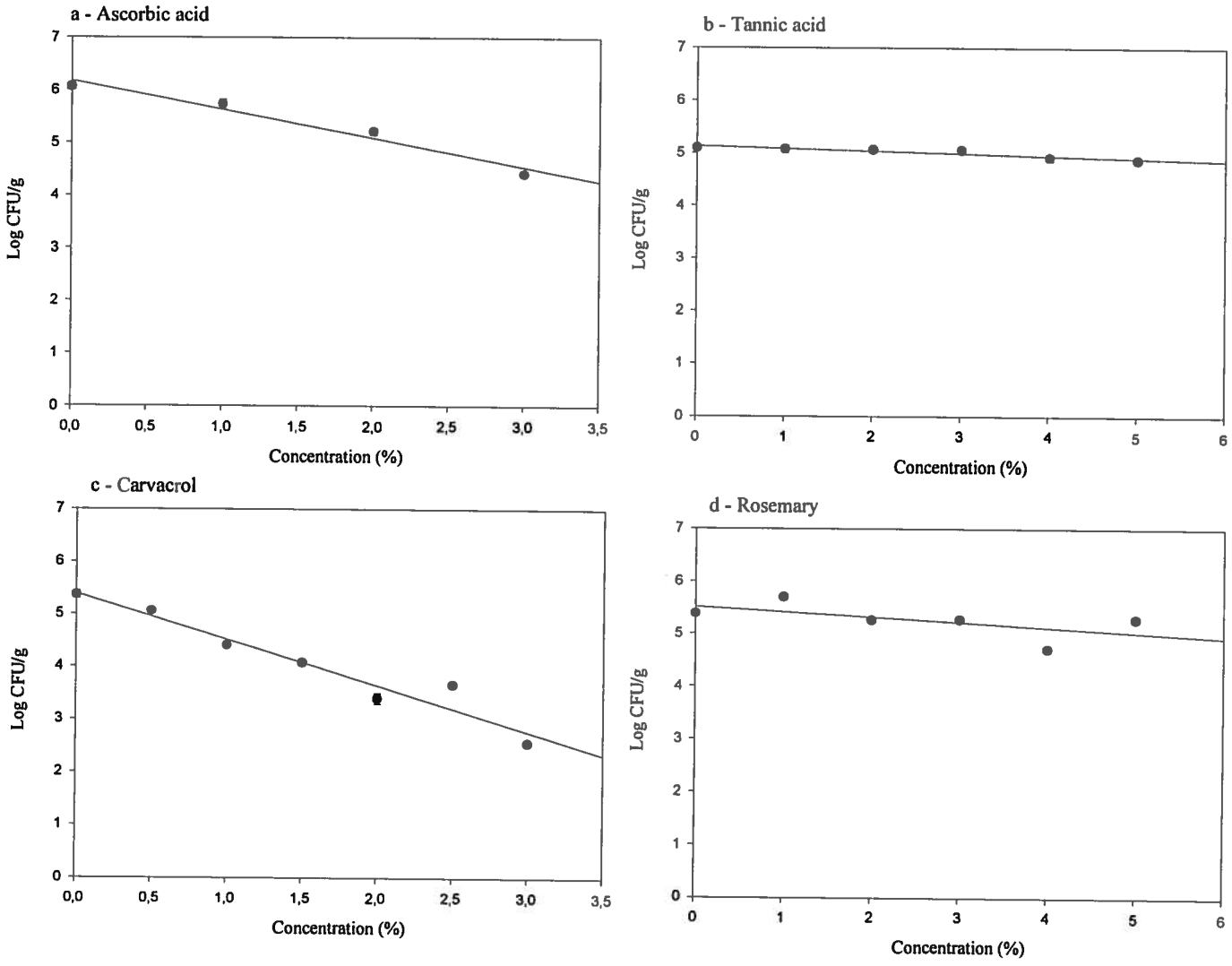


Figure 2 : Antimicrobial effectiveness of different additives on *S. typhi* in ground beef

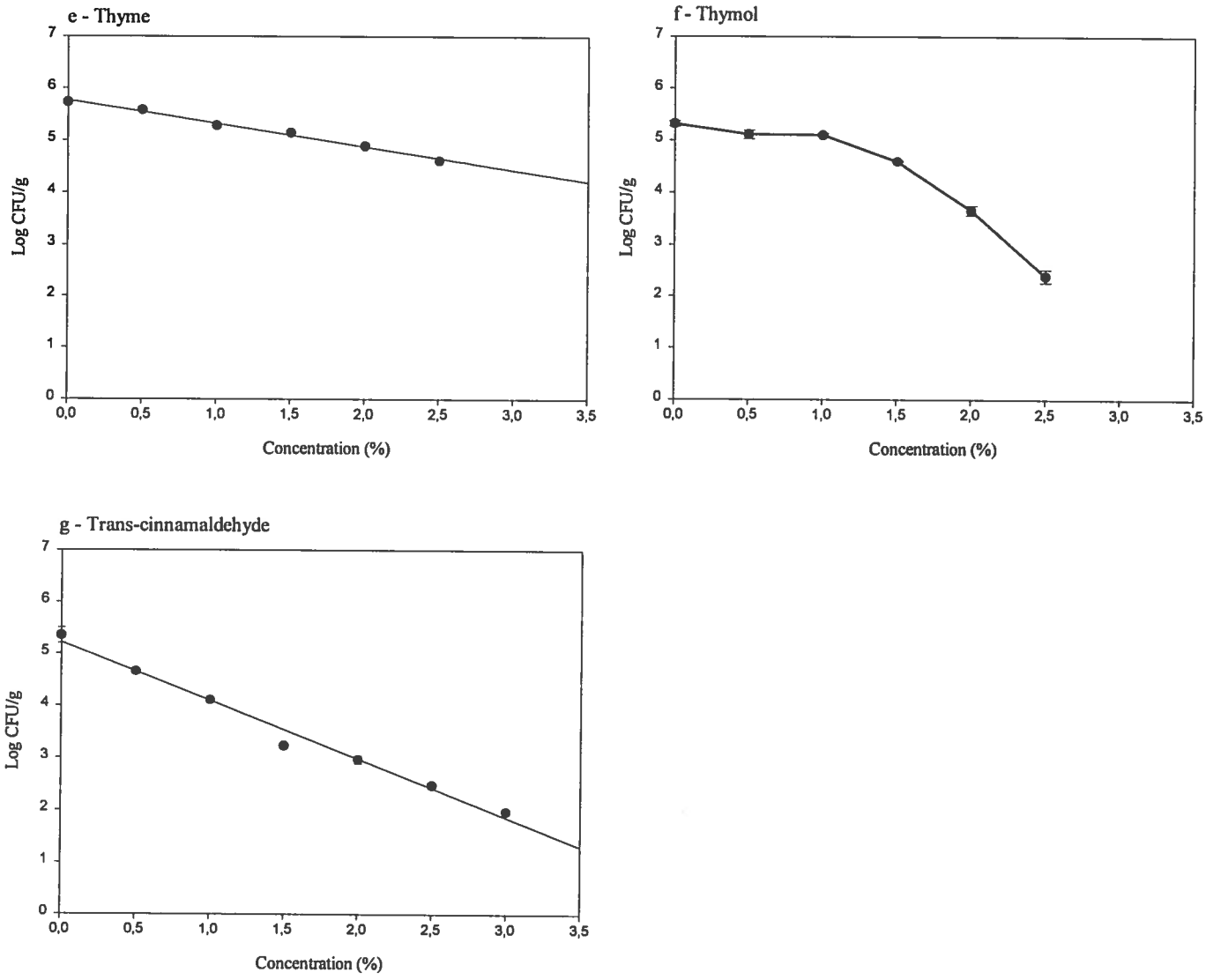


Figure 2 (contd.) : Antimicrobial effectiveness of different additives on *S. typhi* in ground beef

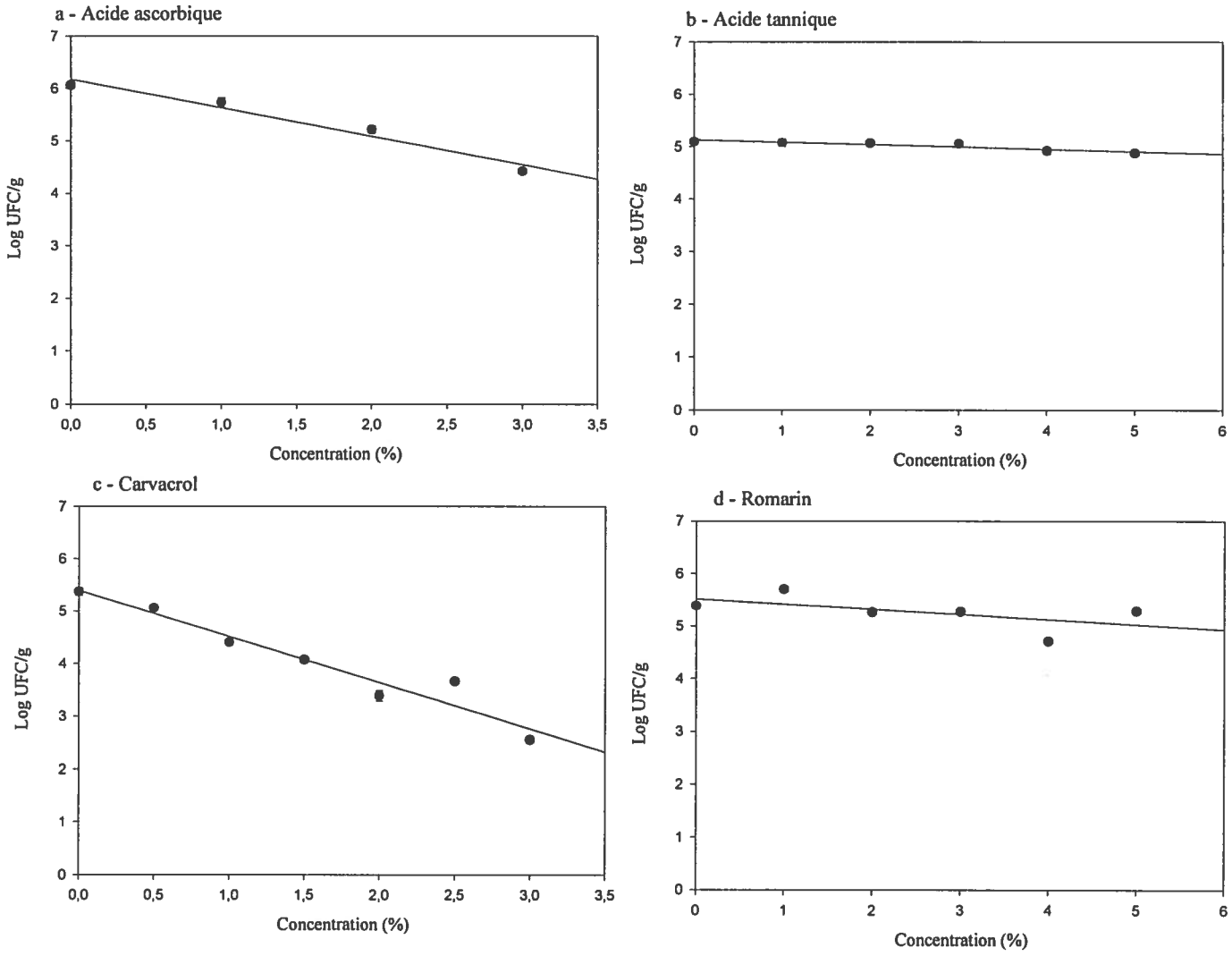


Figure 2a : Efficacité antimicrobienne de différents additifs sur *S. typhi* dans du bœuf haché

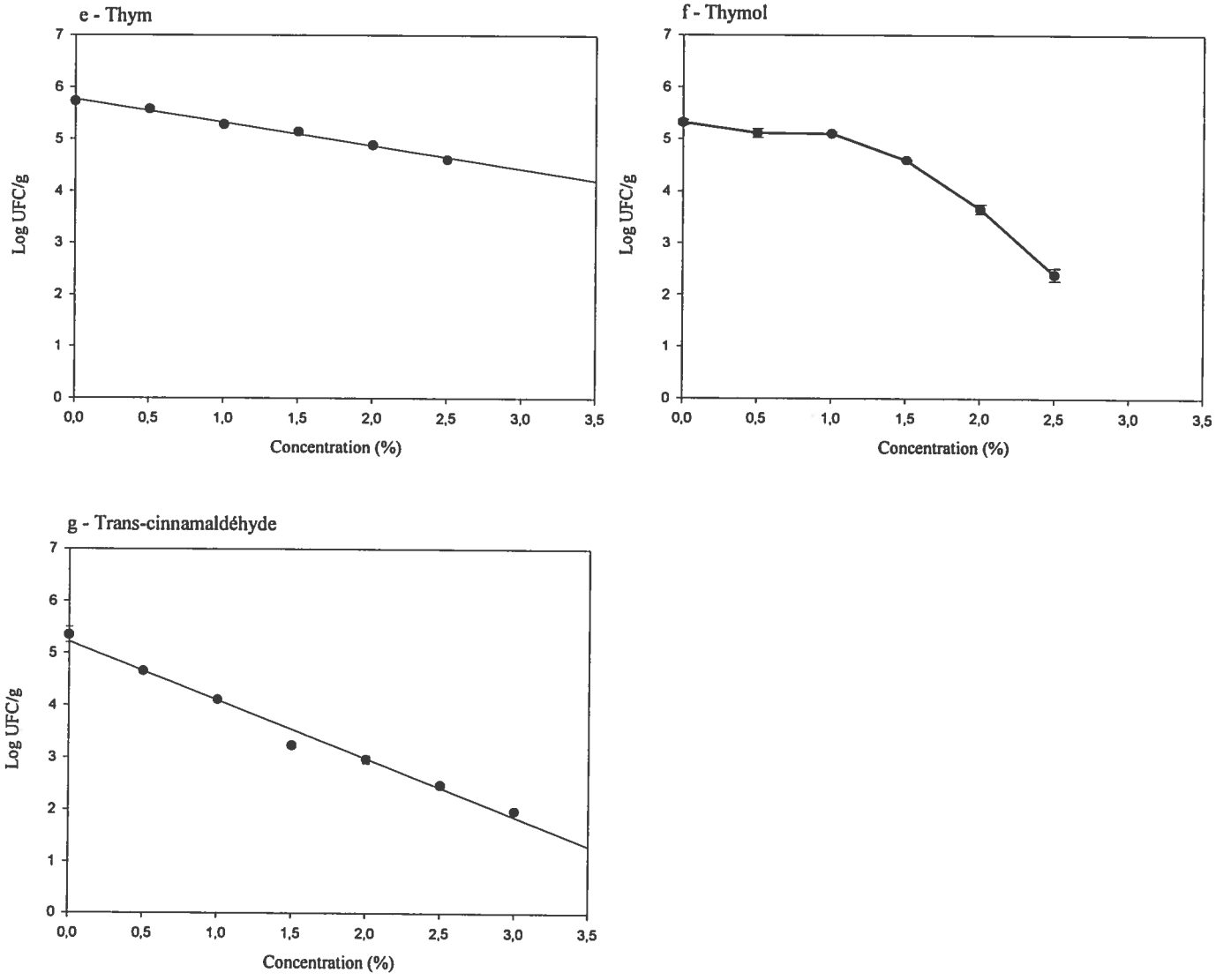


Figure 2a (suite) : Efficacité antimicrobienne de différents additifs sur *S. typhi* dans du bœuf haché

Different types of commercial products of Herbalox and Duralox were included in the evaluation of the MIC needed to reduce by 1 log the bacterial population of *E. coli* in ground beef. Results are summarised in table 3 and figures 3 and 4. As shown in figure 3 and 4, the concentration used to determine the minimal concentration were not enough to produce 1 log reduction. However, an estimation of the minimal concentration was made using the slope of the curve.

According to the results presented in Table 3, the addition of the three types of Duralox and Herbalox demonstrated that concentrations ranging from 3.06 % to 8.70 % respectively were necessary to reduce by 1 log the bacterial population of *E. coli* in ground beef. Of the six products tested, the best result was obtained for Duralox AR Seasoning MFD showing an antimicrobial effect on *E. coli* at a minimal concentration of 3.06 %. Herbalox Type O and Herbalox Type HT25 had the lowest antimicrobial effect. The minimal concentration needed to reduce by 1 log the population of *E. coli* were respectively 8.21 % and 8.70 %. These data showed that the rosemary extract used in our laboratory, with a MIC value of 10.37 %, is less efficient in reducing *E. coli* in ground beef than the commercial version of rosemary, Duralox and Herbalox.

According to the results presented in Table 3, the addition of Duralox and Herbalox can reduce by 1 log the bacterial population of *S. typhi* in ground beef at concentrations from 39.87 % to 72.87 %. With these results, it is possible to conclude that these products have a poor antimicrobial effect on *S. typhi.* Previous work done in our laboratory with a rosemary extract showed that the extract was more efficient than the present commercial version of the product. In our previous work a concentration of 13.56 % of rosemary extract was needed to reduce by 1 log the population of *S. typhi.*

These data showed that the addition of these commercial products to ground beef are less efficient than the other additives, such as carvacrol, thymol, trans-cinnamaldehyde, thyme and ascorbic acid.

Table 3: The estimated minimal concentration of three types of each commercial mixtures of Duralox and Herbalox needed to reduce the bacterial population of *E. coli* and *S. typhi* by 1 log in ground beef

Products[1]	MIC (%)[2]	
	E. coli	*S. typhi*
Duralox AR Seasoning MFD	3.06 ± 0.38^{a}	72.87 ± 5.01^{b}
Herbalox Type HTO	3.45 ± 0.74^{a}	42.92 ± 11.10^{a}
Duralox Oxidation NMC-2	4.21 ± 0.89^{a}	64.33 ± 6.27^{b}
Duralox Oxidation NC-2 Type C	6.30 ± 0.94^{b}	62.00 ± 8.02^{b}
Herbalox Type O	8.21 ± 1.42^{c}	66.29 ± 2.64^{b}
Herbalox Type HT25	8.70 ± 0.12^{c}	39.87 ± 7.06^{a}

[1] Percentage (v/w)

[2] Duncan – [a, b, c,] Values in same columns with different letters are significantly different ($p \leq 0.05$)

Tableau 3a: L'estimation de la concentration minimale de trois types de chacun des trois mélanges commerciaux de Duralox et d'Herbalox nécessaire pour réduire la population bactérienne d'*E. coli* et *S. typhi* d'un log dans du bœuf haché

Produits[1]	MIC (%)[2]	
	E. coli	*S. typhi*
Duralox AR Seasoning MFD	$3,06 \pm 0,38^{a}$	$72,87 \pm 5,01^{b}$
Herbalox Type HTO	$3,45 \pm 0,74^{a}$	$42,92 \pm 11,10^{a}$
Duralox Oxidation NMC-2	$4,21 \pm 0,89^{a}$	$64,33 \pm 6,27^{b}$
Duralox Oxidation NC-2 Type C	$6,30 \pm 0,94^{b}$	$62,00 \pm 8,02^{b}$
Herbalox Type O	$8,21 \pm 1,42^{c}$	$66,29 \pm 2,64^{b}$
Herbalox Type HT25	$8,70 \pm 0,12^{c}$	$39,87 \pm 7,06^{a}$

[a] Pourcentage (v/p)

[2] Duncan [a, b, c, d] Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0.05$)

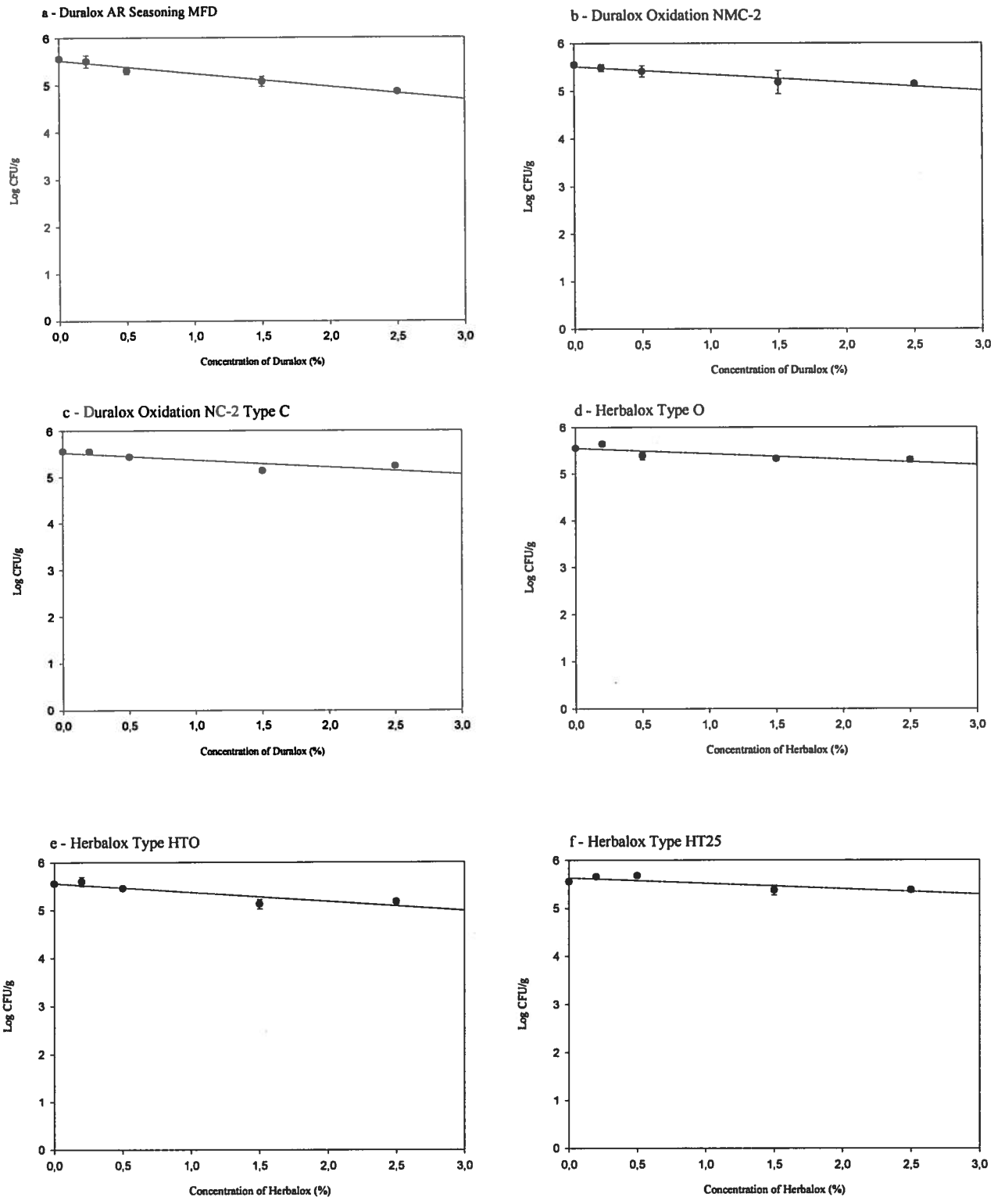


Figure 3 : Effect of Duralox and Herbalox concentration on *E. coli* present in ground beef

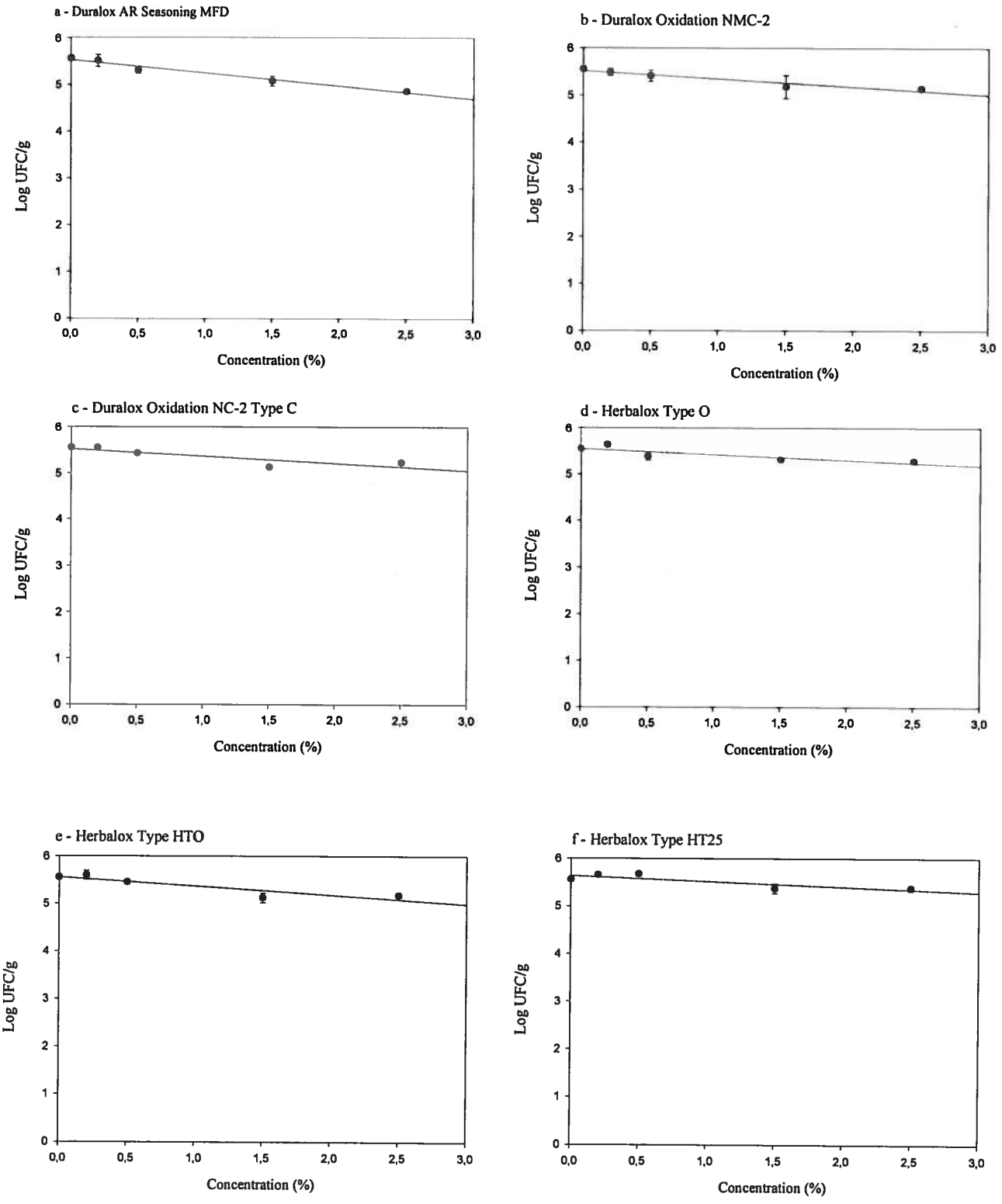


Figure 3a : Effet de la concentration de Duralox et Herbalox sur *E. coli* dans du boeuf haché

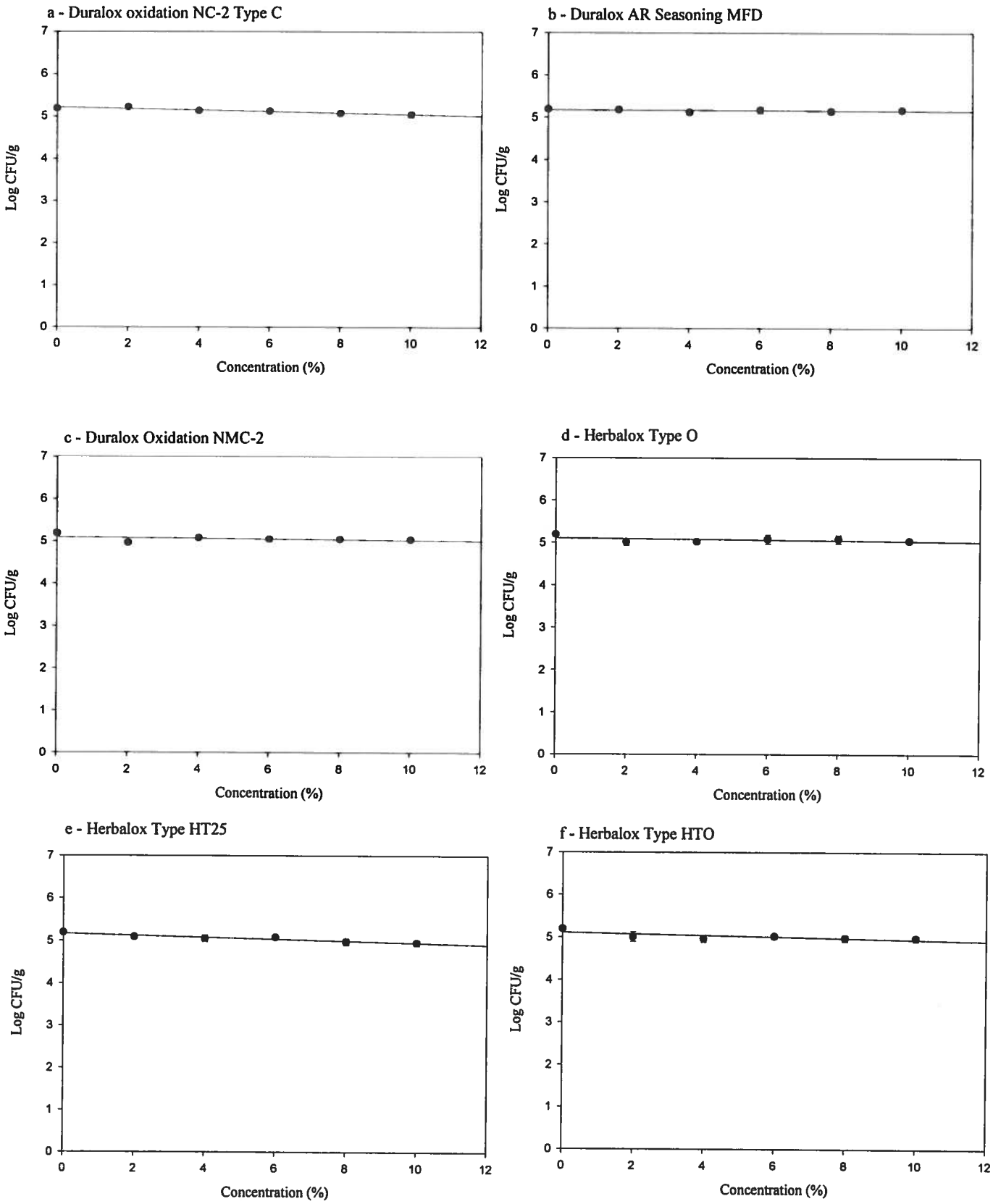


Figure 4 : Effect of Duralox and Herbalox concentration on *S. typhi* present in ground beef

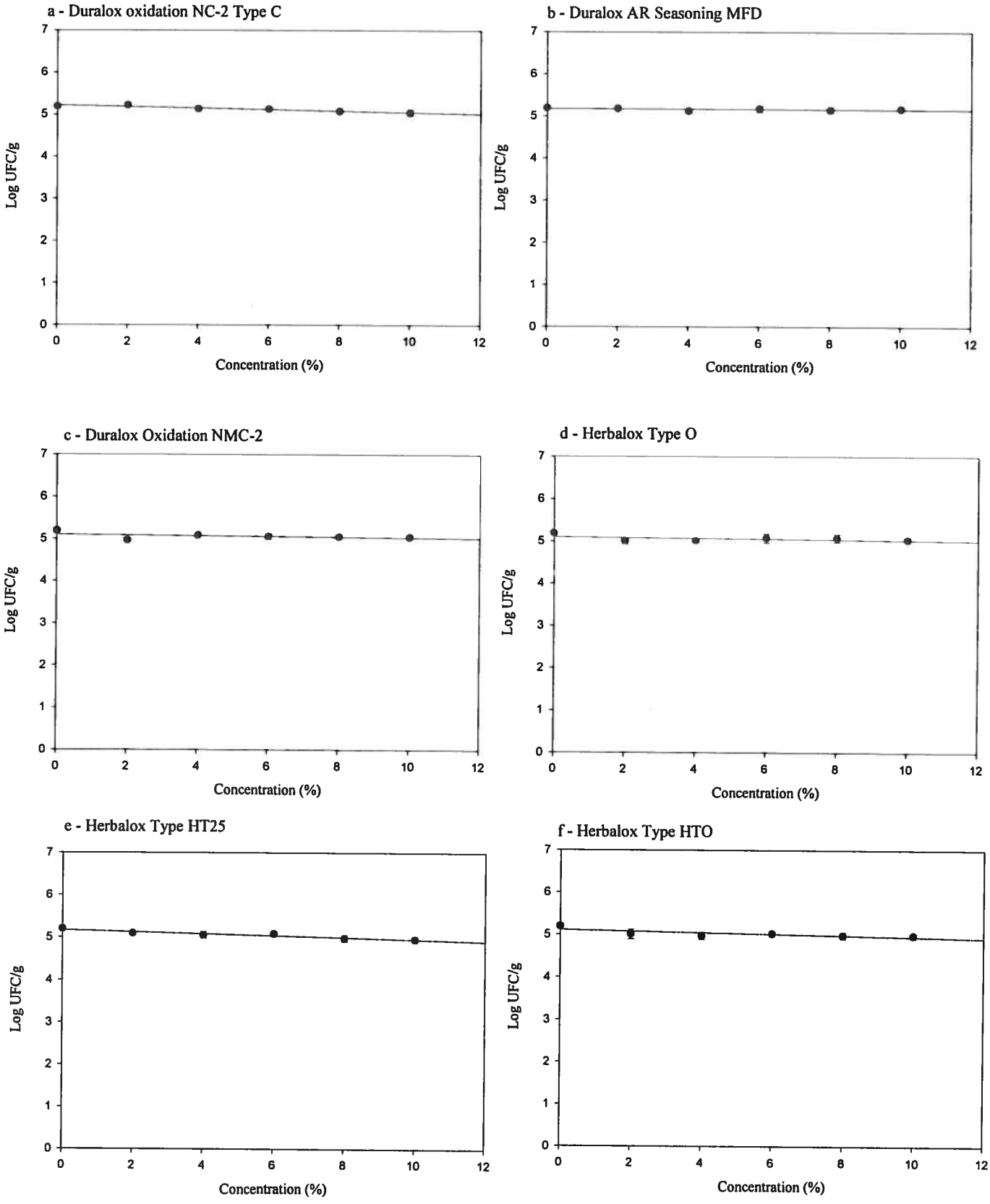


Figure 4a : Concentration nécessaire pour réduire la population bactérienne de *S. typhi* en présence de trois types de Duralox et trois types d'Herbalox dans du boeuf haché

4.2. Irradiation sensitivity of *E. coli* and *S. typhi* in presence of various additives

4.2.1. *Escherichia coli*

Table 4 and figure 5 show the irradiation sensitivity of *E. coli* in ground beef in the presence of various additives. According to table 4, the irradiation sensitivity of *E. coli* was 0.126 kGy. Results showed that the addition of most additives had an effect on the irradiation sensitivity of *E. coli*. The most effective additives were those with the concentration corresponding to the MIC in the ground beef. The addition of trans-cinnamaldehyde (1.5 %) significantly reduced ($p \leq 0.05$) the D_{10}, from 0.126 kGy to 0.037 kGy, indicating a substantial increase in irradiation sensitivity of *E. coli*. The efficiency (defined as the percentage of reduction of the D_{10} value in the presence of an additive compared to the control) of trans-cinnamaldehyde was 70.6 %. This was followed by thymol (1.15 %), thyme (2.33 %) and carvacrol (0.88 %) with D_{10} values of 0.087 kGy, 0.090 kGy and 0.103 kGy, respectively. The efficiency of these additives were 40.0 %, 28.6 % and 18.2 %, respectively.

Even at low concentrations, other additives also significantly increased ($p \leq 0.05$) the irradiation sensitivity of *E. coli*. These additives were thymol (0.1 %); tannic acid (0.38 %); rosemary (0.5 %); BHT (0.01 %); trans-cinnamaldehyde (0.025 %); carvacrol (0.125 %); thyme (0.2 %) and BHA (0.01 %). D_{10} values ranged from 0.103 kGy to 0.117 kGy. The sensitivity of *E. coli* with the addition of these additives was increased by 18.2 % to 7.1 % (Table 4). The addition of nisin (625 UI/g); nisin (625 UI/g) + EDTA (100 ppm); EDTA (100 ppm), tetrasodium pyrophosphate (0.1 %) and carnosine (1.0 %) had no significant effect ($p > 0.05$) on the irradiation sensitivity of *E. coli*. The D_{10} values ranged from 0.120 kGy to 0.133 kGy. Only one of these additive significantly decreased ($p \leq 0.05$) the irradiation sensitivity, ascorbic acid. The addition of ascorbic acid (0.5 %) had a protective effect of 11.9 %, with a D_{10} of 0.141 kGy.

Figure 5 shows that adding almost all of the additives tested under these conditions, the irradiation dose necessary to reduce *E. coli* under the detection level was lower than using irradiation without the addition of additives to the ground beef. In the presence of the additives that helped to increase the lethal effect of irradiation, the irradiation dose necessary to reduce *E. coli* under the detection level ranged from 0.2 kGy to 0.6 kGy, compared to 0.7 kGy for the control. With the addition of ascorbic acid, *E. coli* was undetectable at an irradiation dose of 0.75 kGy. These results suggest that the addition of those additives to the ground beef was able to reduce the irradiation dose necessary to reduce *E. coli* under the detection level from 1.2 times to 3.5 times.

These results suggest that additives have a synergistic effect with irradiation treatment on the sensitivity of *E. coli* to irradiation. Nine of the thirteen additives tested for this important synergistic effect on the irradiation sensitivity of *E. coli* when incorporated in ground beef prior to irradiation. These additives were trans-cinnamaldehyde, thymol, carvacrol, thyme, tannic acid, rosemary, BHT, BHA, nisin and nisin+EDTA. Among the additives tested, trans-cinnamaldehyde (1.5 %) was the most effective, with an increase in irradiation sensitivity corresponding to an efficiency of 70.6 %. Also three other additives (EDTA, tetrasodium pyrophosphate and carnosine) had no effect on *E. coli* and only ascorbic acid increased the irradiation resistance of *E. coli*.

4.2.2. *Salmonella typhi*

Table 5 and figure 6 show the irradiation sensitivity of *S. typhi* in ground beef in the presence of various additives. The D_{10} value of the control was 0.526 kGy. Except for ascorbic acid, all the additives increased the irradiation sensitivity of *S. typhi*, with D_{10} varying from 0.139 to 0.494 kGy. The most effective additives were the ones with the concentration corresponding to the MIC in ground beef. The addition of trans-cinnamldehyde (0.89 %), carvacrol (1.15 %), thymol (1.6 %) and thyme (2.75 %) to ground beef significantly increased ($p \leq 0.05$) the irradiation sensitivity, with D_{10} value of 0.139 kGy, 0.208 kGy, 0.210 kGy and 0.260 kGy respectively. The efficiency of those additives were 73.6 %, 60.4 %, 60.1 % and 50.6 %.

The addition of tannic acid (0.38 %) helped to increase the irradiation sensitivity of *S. typhi* by 42.6 %, with a D_{10} value of 0.302 kGy. The addition of the mixture of nisin (625 UI/g) and EDTA (100 ppm), carvacrol (0.125 %), tetrasodium pyrophosphate (0.1 %) and trans-cinnamaldehyde (0.025 %) helped to significantly increase ($p \leq 0.05$) the irradiation sensitivity of *S. typhi*. D_{10} values were 0.340 kGy, 0.343 kGy, 0.356 kGy and 0.356 kGy respectively. These values represents an efficiency ranging from 35.4 % to 32.3 %. Thymol, at a concentration of 0.1 %, was just as efficient as tetrasodium pyrophosphate and trans-cinnamaldehyde at a concentration of 0.025 %. The D_{10} was 0.362 kGy, representing an increase in sensitivity of 31.2 %. Even at a lower concentration, the addition of thyme at a concentration of 0.2 % also increased the irradiation sensitivity of the bacteria by 26.6 %, and the D_{10} value was 0.386 kGy.

For BHT (0.01%), BHA (0.01 %), EDTA (100 ppm) and nisin (625 UI/g), the D_{10} values were evaluated at 0.405 kGy, 0.407 kGy, 0.419 kGy and 0.420 kGy respectively. The increase of bacterial sensitivity in the presence of these additives ranged from 20.2 % to 23.0 %. When combining nisin (625 UI/g) with EDTA (100 ppm), the D_{10} value was reduced from 0.526 kGy to 0.340 kGy, meaning an increase in efficiency 35.4 % compared to 20.3 % for EDTA (100 ppm) alone and 20.2 % for nisin (625 UI/g) alone. The addition of rosemary (0.5 %) was just as efficient as EDTA (100 ppm) and nisin (625 UI/g). The D_{10} value was 0.436 kGy, representing an increase in sensitivity of 17.1 %. Finally, the addition of carnosine (1.0 %) helped to increase the irradiation sensitivity by only 6.1 %, with a D_{10} value of 0.494 kGy.

Only one additives had no significant effect ($p > 0.05$) on the irradiation sensitivity of *S. typhi*. The addition of ascorbic acid (0.5 %) to the ground beef did not affect the D_{10} value, which was 0.521 kGy.

The addition of most of these additives to the ground beef reduced the irradiation dose necessary to reduce *S. typhi* under the detection level. Without any additive, a dose of 2.9 kGy was necessary to reduce *S. typhi* under the detection level present in ground beef. In the presence of trans-cinnamaldehyde (0.89 %), carvacrol (1.15 %), thymol (1.6 %) or thyme (2.75 %), the bacteria was undetectable at doses of 0.75 kGy, 1.3 kGy, 1.3 kGy and 1.55 kGy, respectively. In the presence of the other additives tested, the irradiation doses necessary to decrease the population of *S. typhi* under the detection level from the ground beef ranged from 1.55 kGy to 2.6 kGy.

These results suggest a killing contribution between irradiation and the additives on the sensitivity of *S. typhi*. The addition of those additives to ground beef was able to reduce the irradiation dose necessary to completely eliminate *S. typhi* by 1.1 times to 3.9 times. This means that except for ascorbic acid all of the additives tested had a synergistic effect on the irradiation sensitivity of *S. typhi* when incorporated in ground beef prior to irradiation. Among the additives tested, trans-cinnamaldehyde (1.5 %) was the most effective, with an efficiency of 73.6 %, and only ascorbic acid had no effect on *S. typhi*.

Comparing results obtained with *E. coli* and *S. typhi*, it is possible to note that *S. typhi* was more resistant to the irradiation treatment, D_{10} value being 0.126 kGy and 0.526 kGy respectively in the absence of additive. The addition of the various additives tested affected the sensibility of both bacteria to irradiation. The addition of carvacrol, thyme, thymol and trans-cinnamaldehyde at the respective MICs in ground beef was more efficient in increasing the irradiation sensitivity of *E. coli* and *S. typhi* than at the MICs in broth. The increase of sensitivity was proportional to the concentration of the additives. However, the increase in sensitivity was greater with *S. typhi* than with *E. coli*, with efficiency percentages higher for *S. typhi*.

Table 4 : Irradiation sensitivity of *E. coli* in ground beef in presence of additives

Additives[1]	Properties[2]	D_{10} (kGy)[3]	Efficiency[4]
Control		0.126 ± 0.0036^{gh}	
Trans-cinnamaldehyde (1.5 %)[b]	A	0.037 ± 0.0012^{a}	70.6 %
Thymol (1.15 %)[b]	A	0.087 ± 0.0036^{b}	40.0 %
Thyme (2.33 %)[b]	A	0.090 ± 0.0036^{b}	28.6 %
Carvacrol (0.88 %)[b]	A	0.103 ± 0.0027^{c}	18.2 %
Thymol (0.1 %)[a]	A	0.103 ± 0.0094^{c}	18.2 %
Tannic acid (0.38 %)[d]	AB	0.106 ± 0.0012^{cd}	15.9 %
Rosemary (0.5 %)[a]	B	0.111 ± 0.0035^{de}	11.9 %
BHT (0.01 %)[c]	B	0.115 ± 0.0020^{ef}	8.7 %
Trans-cinnamaldehyde (0.025 %)[a]	A	0.115 ± 0.0041^{ef}	8.7 %
Carvacrol (0.125 %)[a]	A	0.115 ± 0.0036^{ef}	8.7 %
Thyme (0.2 %)[a]	A	0.117 ± 0.0147^{ef}	7.1 %
BHA (0.01 %)[c]	B	0.117 ± 0.0026^{ef}	7.1 %
Nisin (625 UI/g)[b]	A	0.120 ± 0.0089^{efg}	4.8 %
Nisin (625 UI/g)[b] + EDTA (100 ppm)[c]	A + ABC	0.121 ± 0.0066^{fg}	4.0 %
EDTA (100 ppm)[c]	ABC	0.127 ± 0.0033^{gh}	-0.8 %
Tetrasodium pyrophosphate (0.1 %)[c]	D	0.131 ± 0.0079^{h}	-4.0 %
Carnosine (1.0 %)[e]	BE	0.133 ± 0.0075^{h}	-5.6 %
Ascorbic acid (0.5 %)[f]	BE	0.141 ± 0.0068^{i}	-11.9 %

[1] [a] MIC of additives present in artificial culture media to reduce by 1 log six pathogenic and spoilage bacteria

[b] MIC of additives in ground beef

[c] Concentration recommended by the Canadian Food Inspection Agency (CFIA)

[d] Concentration from literature

[e] Concentration from literature (Sebranek, 1999)

[f] Concentration from literature (Giroux, 2000)

[2] A : antimicrobial properties; B : antioxidant properties; C : chelator; D : moisture retention properties; E : colour stabiliser

[3] Duncan - [a,b,c,d,e,f,g,h,i,j] Values in same columns with different letters are significantly different ($p \leq 0.05$)

[4] The values with a " - " have a protective effect on the bacteria compared with the control

Tableau 4a : Sensibilité de *E. coli* à l'irradiation en présence de différents additifs dans du bœuf haché

Additifs[1]	Propriétés[2]	D_{10} (kGy)[3]	Efficacité[4]
Témoin		$0,126 \pm 0,0036^{gh}$	
Trans-cinnamaldéhyde (1,5 %)[b]	A	$0,037 \pm 0,0012^{a}$	70,6 %
Thymol (1,15 %)[b]	A	$0,087 \pm 0,0036^{b}$	40,0 %
Thym (2,33 %)[b]	A	$0,090 \pm 0,0036^{b}$	28,6 %
Carvacrol (0,88 %)[b]	A	$0,103 \pm 0,0027^{c}$	18,2 %
Thymol (0,1 %)[a]	A	$0,103 \pm 0,0094^{c}$	18,2 %
Acide tannique (0,38 %)[d]	AB	$0,106 \pm 0,0012^{cd}$	15,9 %
Romarin (0,5 %)[a]	B	$0,111 \pm 0,0035^{de}$	11,9 %
BHT (0,01 %)[c]	B	$0,115 \pm 0,0020^{ef}$	8,7 %
Trans-cinnamaldéhyde (0,025 %)[a]	A	$0,115 \pm 0,0041^{ef}$	8,7 %
Carvacrol (0,125 %)[a]	A	$0,115 \pm 0,0036^{ef}$	8,7 %
Thym (0,2 %)[a]	A	$0,117 \pm 0,0147^{ef}$	7,1 %
BHA (0,01 %)[c]	B	$0,117 \pm 0,0026^{ef}$	7,1 %
Nisine (625 UI/g)[b]	A	$0,120 \pm 0,0089^{efg}$	4,8 %
Nisine (625 UI/g)[b] + EDTA (100 ppm)[c]	A + ABC	$0,121 \pm 0,0066^{fg}$	4,0 %
EDTA (100 ppm)[c]	ABC	$0,127 \pm 0,0033^{gh}$	-0,8 %
Tétrasodium pyrophosphate (0,1 %)[c]	D	$0,131 \pm 0,0079^{h}$	-4,0 %
Carnosine (1,0 %)[e]	BE	$0,133 \pm 0,0075^{h}$	-5,6 %
Acide ascorbique (0,5 %)[f]	BE	$0,141 \pm 0,0068^{i}$	-11,9 %

[1] [a] CMI des additifs dans un milieu artificielle contre six bactéries pathogène et de détérioration

[b] CMI des additifs dans le boeuf haché

[c] Concentration recommendée par Canadian Food Inspection Agency (CFIA)

[d] Concentration provenant de littérature

[e] Concentration provenant de la littérature (Sebranek, 1999)

[f] Concentration provenant de la littérature (Giroux, 2000)

[2] A : propriété antimicrobienne; B : propriété antioxydante; C : chelateur; D propriété de rétention d'eau; E : stabilisateur de couleur

[3] Duncan - ^{a,b,c,d,e,f,g,h,i,j} Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0,05$)

[4] Les valeurs avec un " - " ont un effet protecteur sur la bactérie comparativement au témoin

Table 5 : Irradiation sensitivity of *S. typhi* in ground beef in presence of additives

Additives1	Properties[2]	D_{10} (kGy)[3]	Efficiency
Control		0.526 ± 0.0161^{k}	
Trans-cinnamaldehyde (0.89 %)[b]	A	0.139 ± 0.0025^{a}	73.6 %
Carvacrol (1.15 %)[b]	A	0.208 ± 0.0062^{b}	60.4 %
Thymol (1.6 %)[b]	A	0.210 ± 0.0086^{b}	60.1 %
Thyme (2.75 %)[b]	A	0.260 ± 0.0078^{c}	50.6 %
Tannic acid (0.38 %)[d]	AB	0.302 ± 0.0080^{d}	42.6 %
Nisin (625 UI/g)[b] + EDTA (100 ppm)[c]	A + ABC	0.340 ± 0.0118^{e}	35.4 %
Carvacrol (0.125 %)[b]	A	0.343 ± 0.0089^{e}	34.8 %
Tetrasodium pyrophosphate (0.1 %)[c]	D	0.356 ± 0.0126^{ef}	32.3 %
Trans-cinnamaldehyde (0.025 %)[a]	A	0.356 ± 0.0047^{ef}	32.3 %
Thymol (0.1 %)[a]	A	0.362 ± 0.0125^{f}	31.2 %
Thyme (0.2 %)[a]	A	0.386 ± 0.0093^{g}	26.6 %
BHT (0.01 %)[c]	B	0.405 ± 0.0074^{h}	23.0 %
BHA (0.01 %)[c]	B	0.407 ± 0.0123^{h}	22.6 %
EDTA (100 ppm)[c]	ABC	0.419 ± 0.0198^{hi}	20.3 %
Nisin (625 UI/g)[b]	A	0.420 ± 0.0040^{hi}	20.2 %
Rosemary (0.5 %)[a]	B	0.436 ± 0.0083^{i}	17.1 %
Carnosine (1.0 %)[e]	BE	0.494 ± 0.0246^{j}	6.1 %
Ascorbic acid (0.5 %)[f]	BE	0.521 ± 0.0167^{k}	1.0 %

[1] [a] MIC of additives present in artificial culture media to reduce by 1 log six pathogenic and spoilage bacteria

[b] MIC of additives in ground beef

[c] Concentration recommended by the Canadian Food Inspection Agency (CFIA)

[d] Concentration from literature

[e] Concentration from literature (Sebranek, 1999)

[f] Concentration from literature (Giroux, 2000)

[2] A : antimicrobial properties; B : antioxidant properties; C : chelator; D : moisture retention properties; E : colour stabiliser

[3] Duncan - ^{a,b,c,d,e,f,g,h,i,j,k,l} Values in same columns with different letters are significantly different ($p \leq 0.05$)

Tableau 5a : Sensibilité de *S. typhi* à l'irradiation en présence de différents additifs dans du bœuf haché

Additifs[1]	Propriétés[2]	D_{10} (kGy)[3]	Efficacité
Témoin		$0,526 \pm 0,0161^{k}$	
Trans-cinnamaldéhyde (0,89 %)[b]	A	$0,139 \pm 0,0025^{a}$	73,6 %
Carvacrol (1,15 %)[b]	A	$0,208 \pm 0,0062^{b}$	60,4 %
Thymol (1,6 %)[b]	A	$0,210 \pm 0,0086^{b}$	60,1 %
Thym (2,75 %)[b]	A	$0,260 \pm 0,0078^{c}$	50,6 %
Acide tannique (0,38 %)[d]	AB	$0,302 \pm 0,0080^{d}$	42,6 %
Nisine (625 UI/g)[b] + EDTA (100 ppm)[c]	A + ABC	$0,340 \pm 0,0118^{e}$	35,4 %
Carvacrol (0,125 %)[a]	A	$0,343 \pm 0,0089^{e}$	34,8 %
Tétrasodium pyrophosphate (0,1 %)[c]	D	$0,356 \pm 0,0126^{ef}$	32,3 %
Trans-cinnamaldéhyde (0,025 %)[a]	A	$0,356 \pm 0,0047^{ef}$	32,3 %
Thymol (0,1 %)[a]	A	$0,362 \pm 0,0125^{f}$	31,2 %
Thym (0,2 %)[a]	A	$0,386 \pm 0,0093^{g}$	26,6 %
BHT (0,01 %)[c]	B	$0,405 \pm 0,0074^{h}$	23,0 %
BHA (0,01 %)[c]	B	$0,407 \pm 0,0123^{h}$	22,6 %
EDTA (100 ppm)[c]	ABC	$0,419 \pm 0,0198^{hi}$	20,3 %
Nisine (625 UI/g)[b]	A	$0,420 \pm 0,0040^{hi}$	20,2 %
Romarin (0,5 %)[a]	B	$0,436 \pm 0,0083^{i}$	17,1 %
Carnosine (1,0 %)[e]	BE	$0,494 \pm 0,0246^{j}$	6,1 %
Acide ascorbique (0,5 %)[f]	BE	$0,521 \pm 0,0167^{k}$	1,0 %

[1] [a] CMI des additifs dans un milieu artificielle contre six bactéries pathogène et de détérioration

[b] CMI des additifs dans le boeuf haché

[c] Concentration recommendée par Canadian Food Inspection Agency (CFIA)

[d] Concentration provenant de littérature

[e] Concentration provenant de la littérature (Sebranek, 1999)

[f] Concentration provenant de la littérature (Giroux, 2000)

[2] A : propriété antimicrobienne; B : propriété antioxydante; C : chelateur; D propriété de rétention d'eau; E : stabilisateur de couleur

[3] Duncan - ^{a,b,c,d,e,f,g,h,i,j,k,l} Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0,05$)

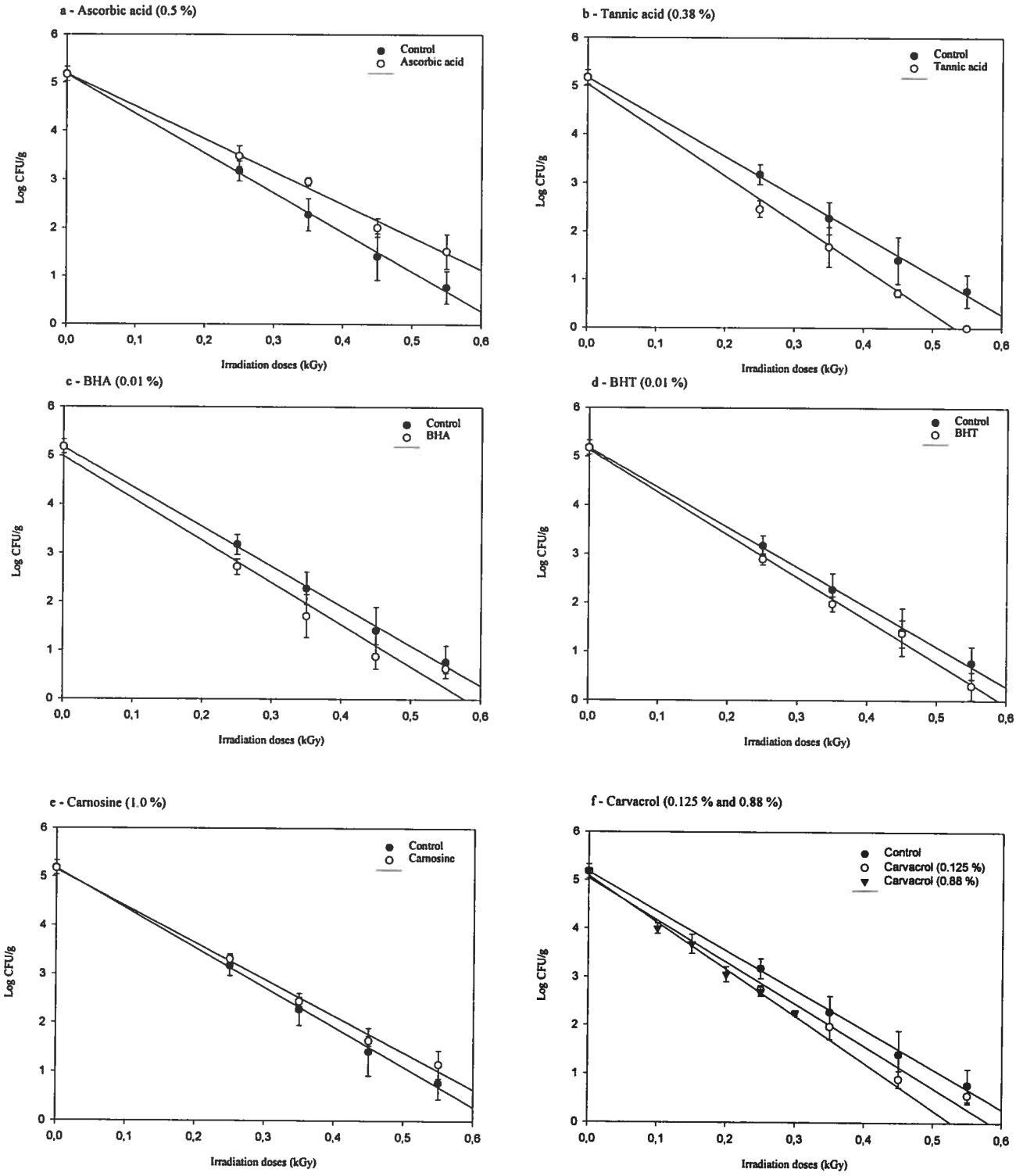
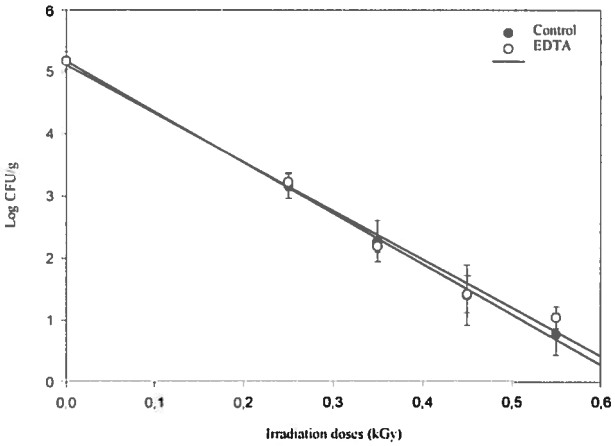
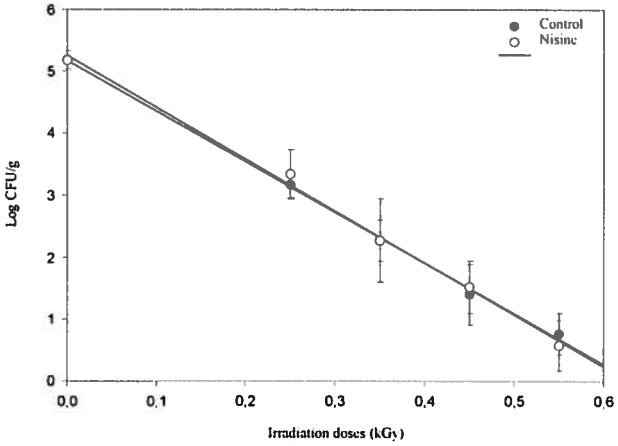


Figure 5 : Irradiation sensitivity of *E. coli* in ground beef in the presence of active compounds

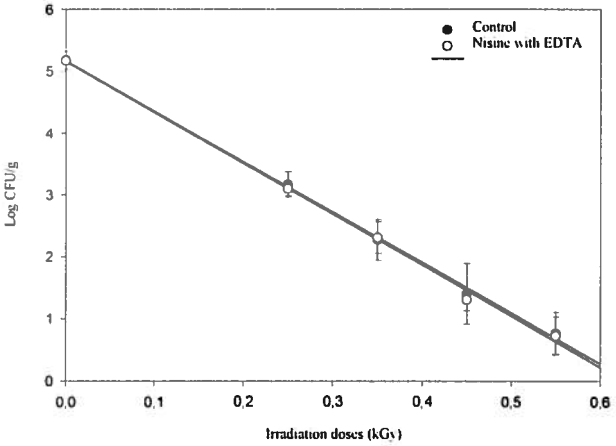
g - EDTA (100 ppm)



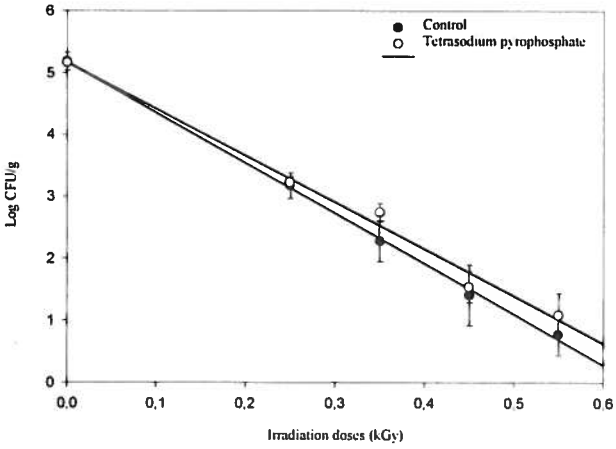
h - Nisin (625 UI/g)



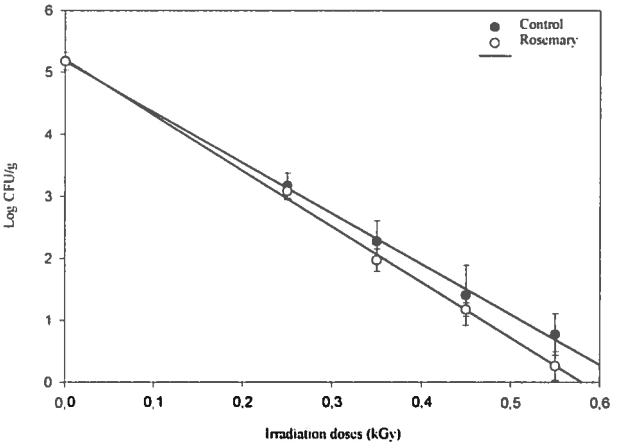
i - Nisin (625 UI/g) with EDTA (100 ppm)



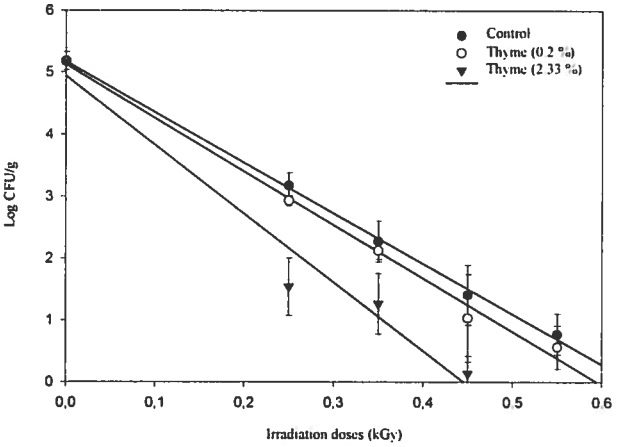
j - Tetrasodium pyrophosphate (0.1 %)



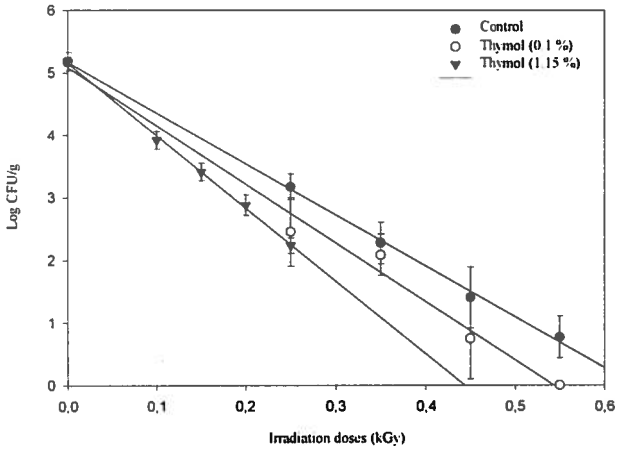
k - Rosemary (0.5 %)



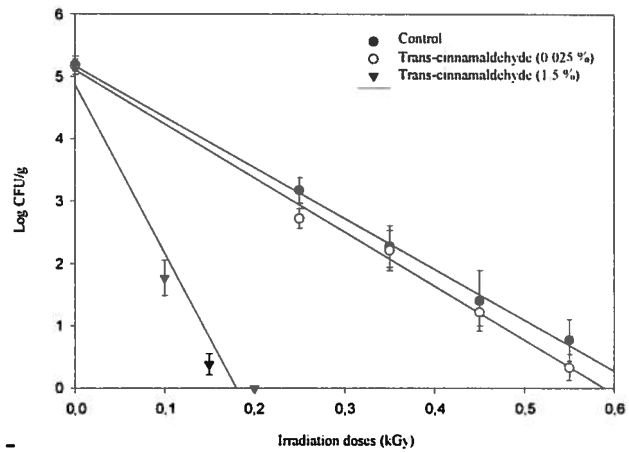
l - Thyme (0.2 % and 2.33 %)



m - Thymol (0.1 % and 1.15 %)



n - Trans-cinnamaldehyde (0.025 % and 1.5 %)



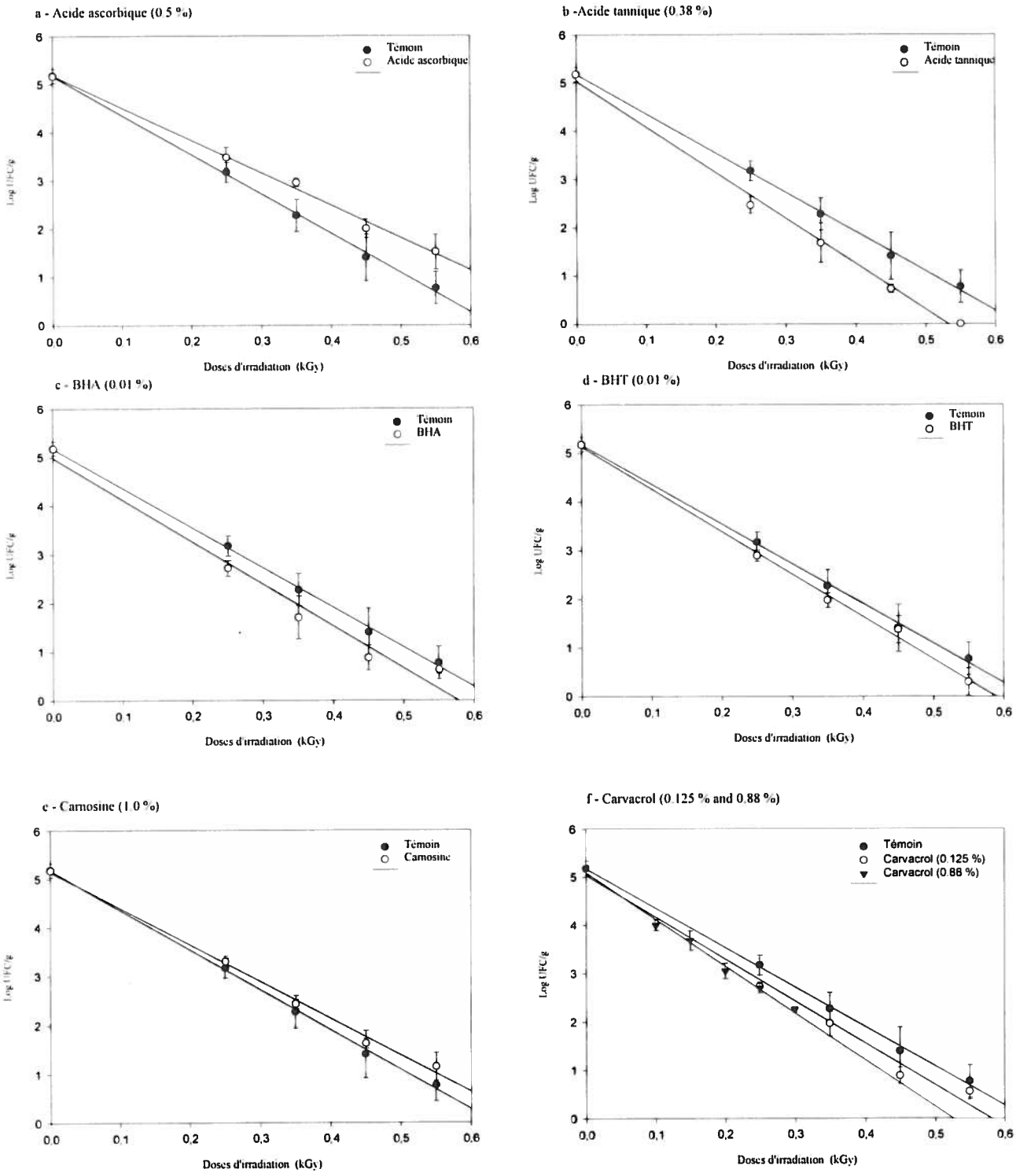
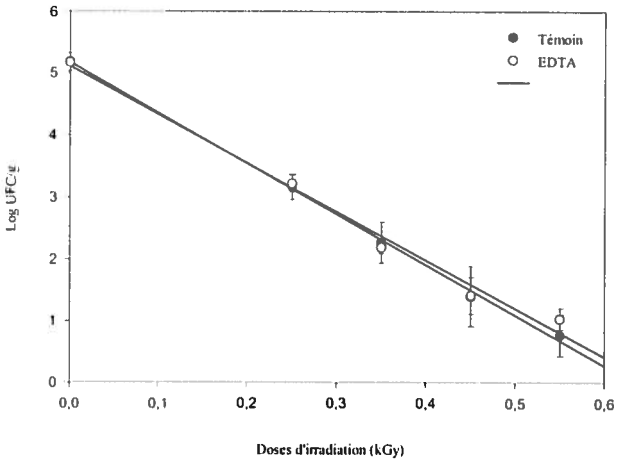
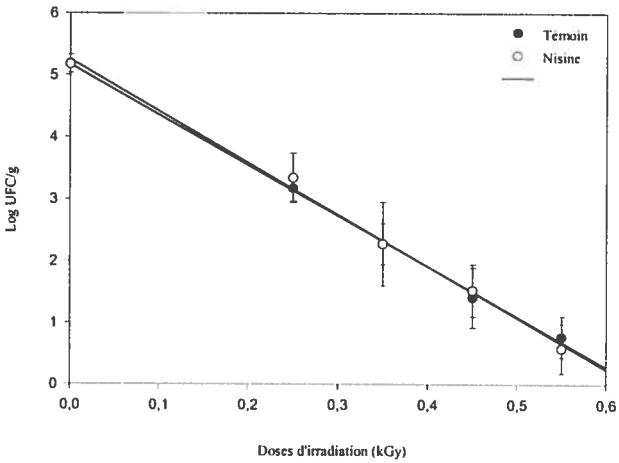


Figure 5a : Sensibilité de *E. coli* à l'irradiation en présence de composés actifs dans du boeuf haché

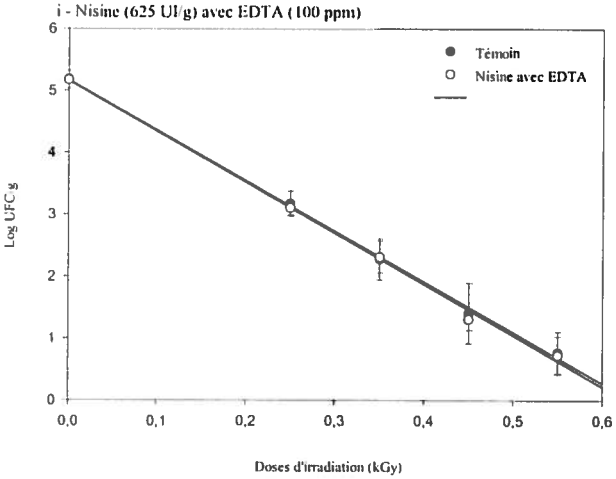
g - EDTA (100 ppm)



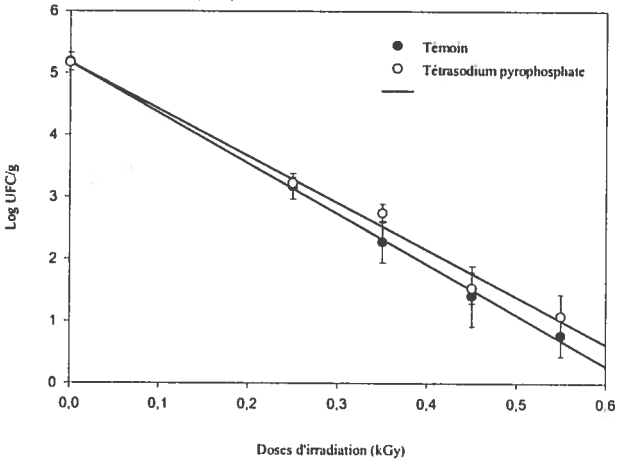
h - Nisine (625 UI/g)



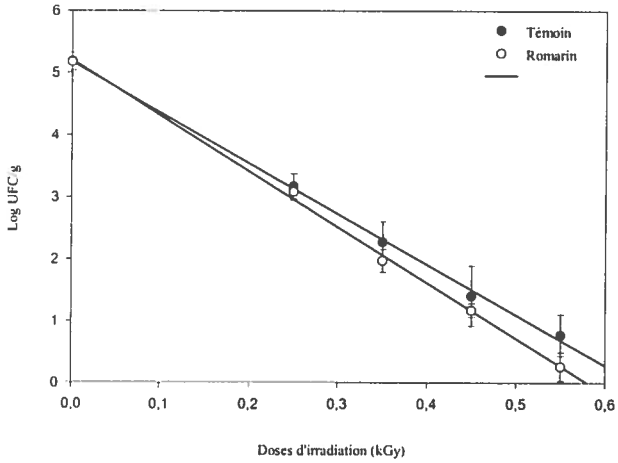
i - Nisine (625 UI/g) avec EDTA (100 ppm)



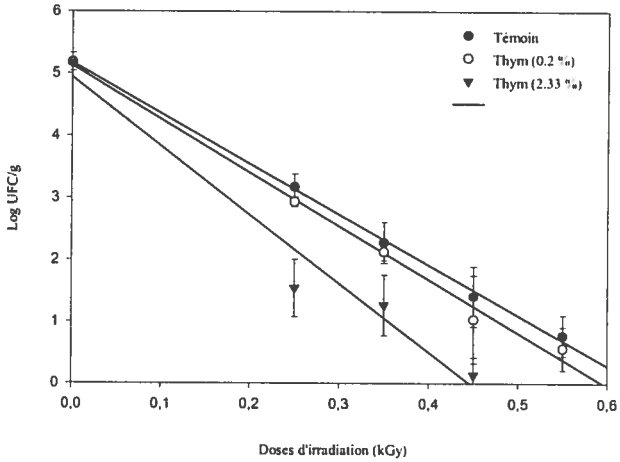
j - Tétrasodium pyrophosphate (0.1 %)



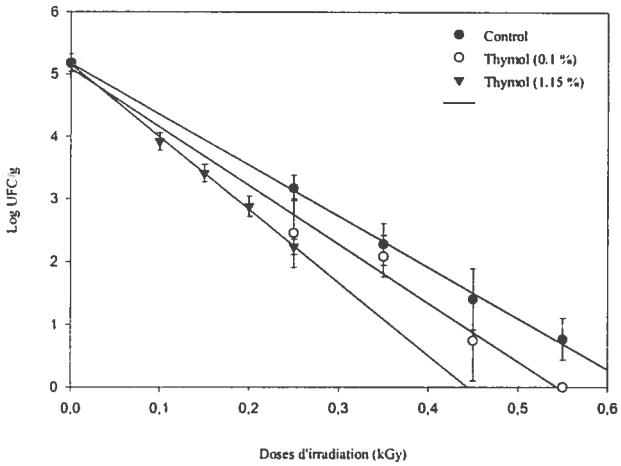
k - Romarin (0.5 %)



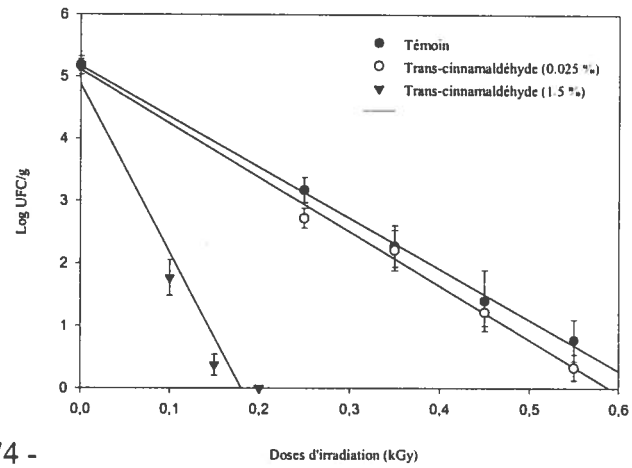
l - Thym (0.2 % et 2.33 %)



m - Thymol (0.1 % et 1.15 %)



n - Trans-cinnamaldéhyde (0.025 % et1.5 %)



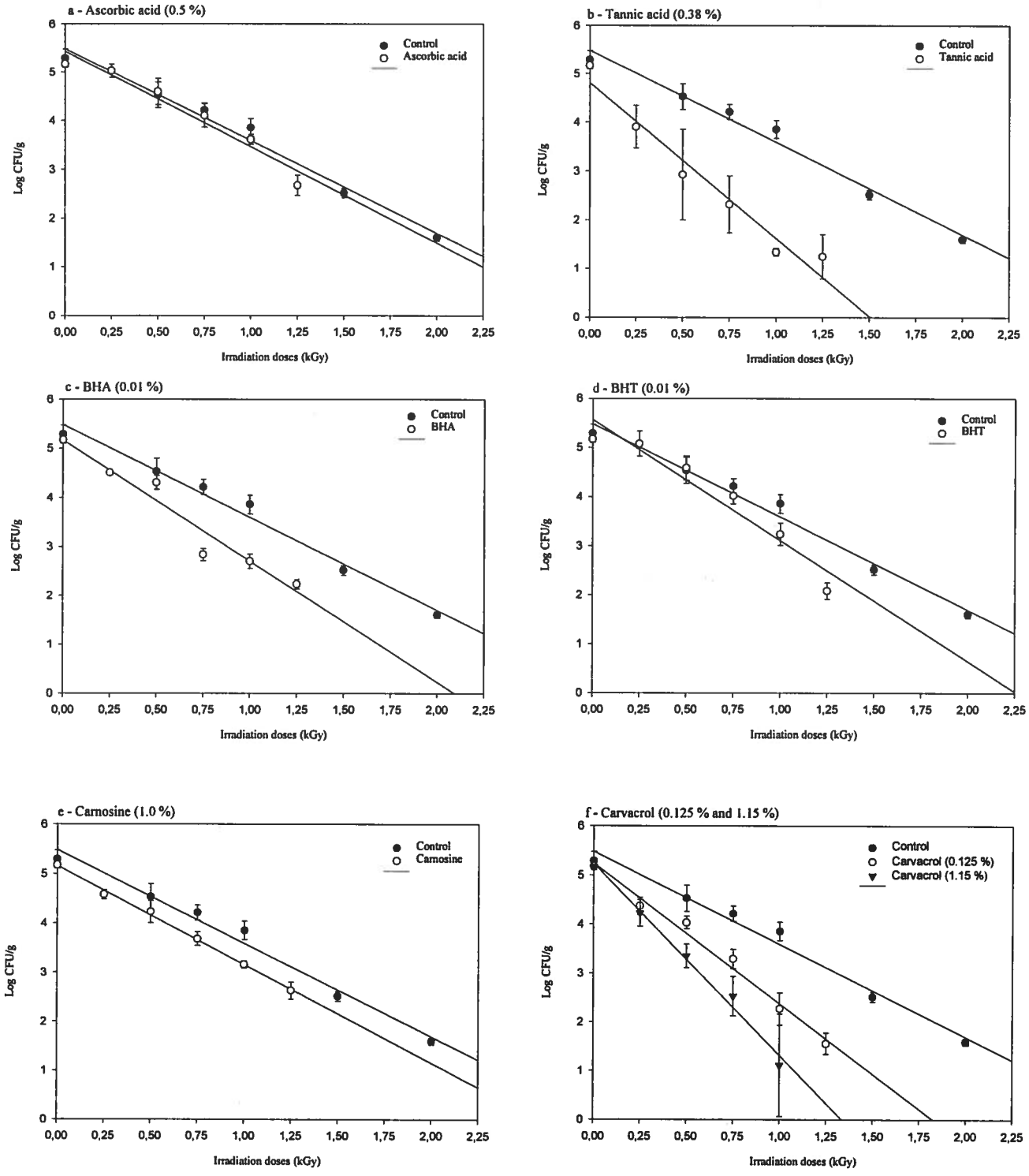
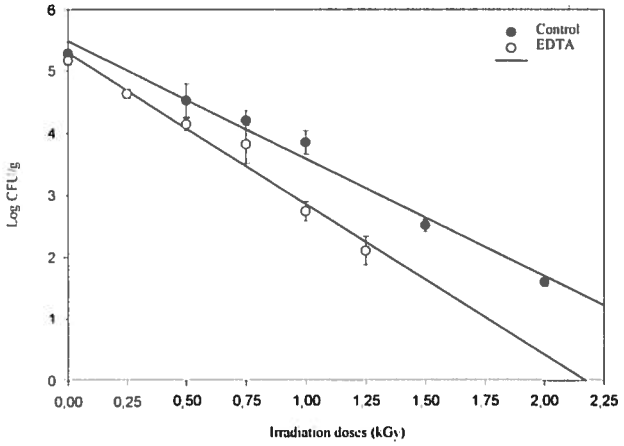
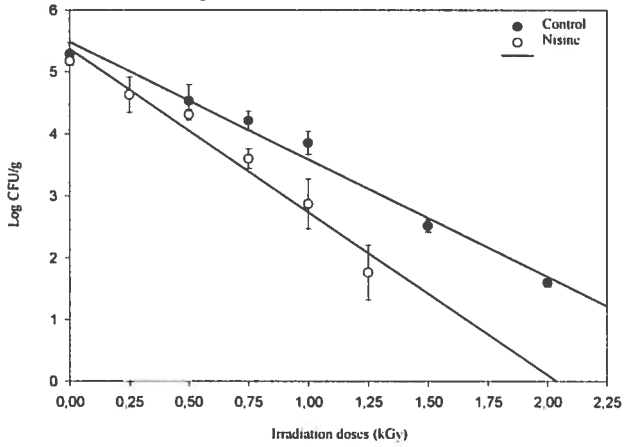


Figure 6 : Irradiation sensitivity of *S.typhi* in ground beef in the presence of active compounds

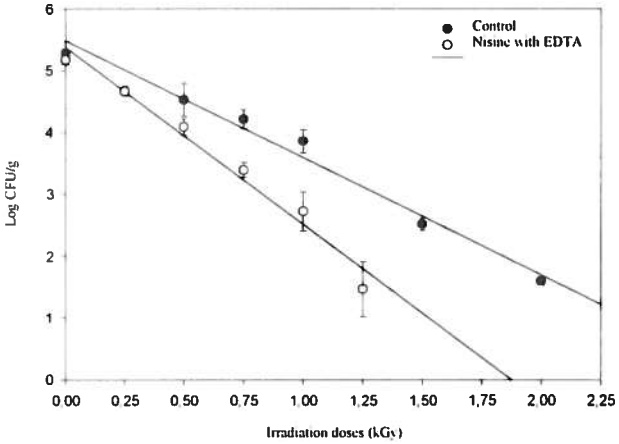
g - EDTA (100 ppm)



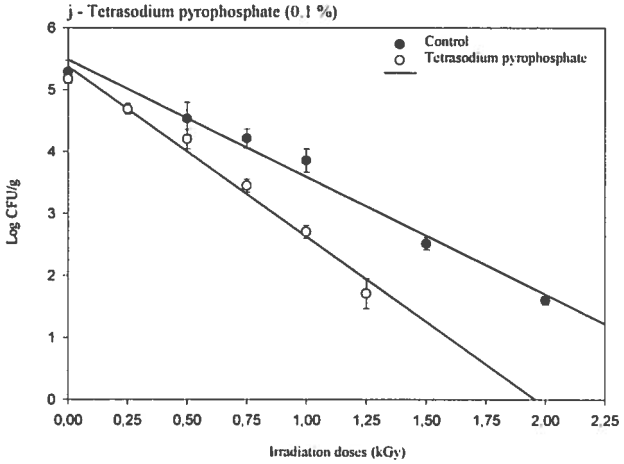
h - Nisin (625 UI/g)



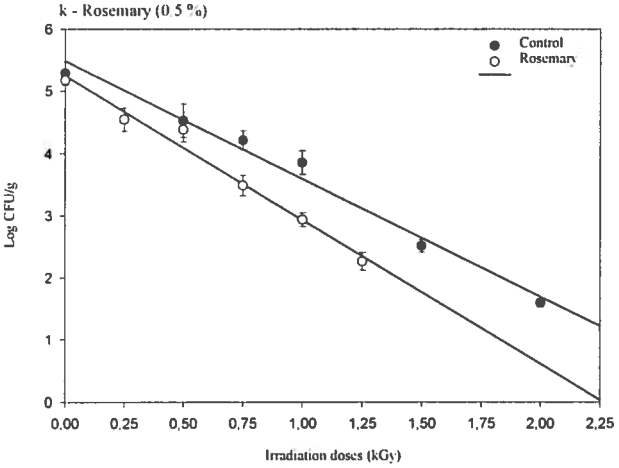
i - Nisin (625 UI/g) with EDTA (100 ppm)



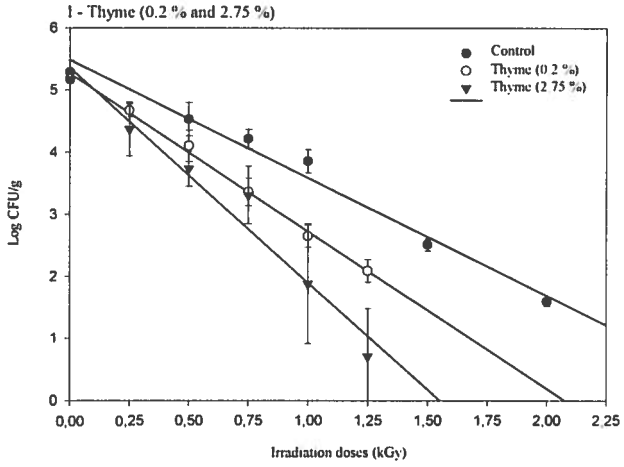
j - Tetrasodium pyrophosphate (0.1 %)



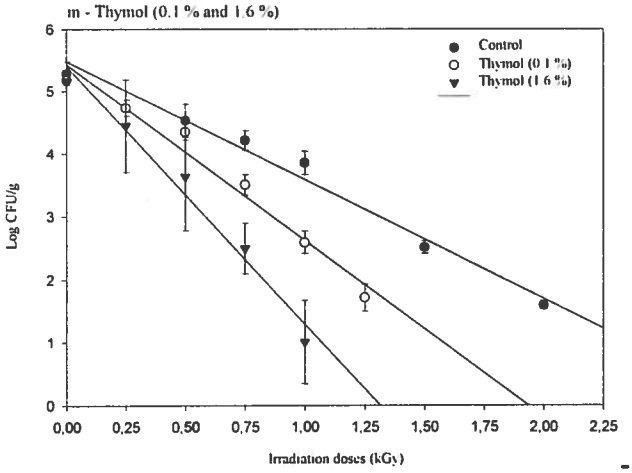
k - Rosemary (0.5 %)



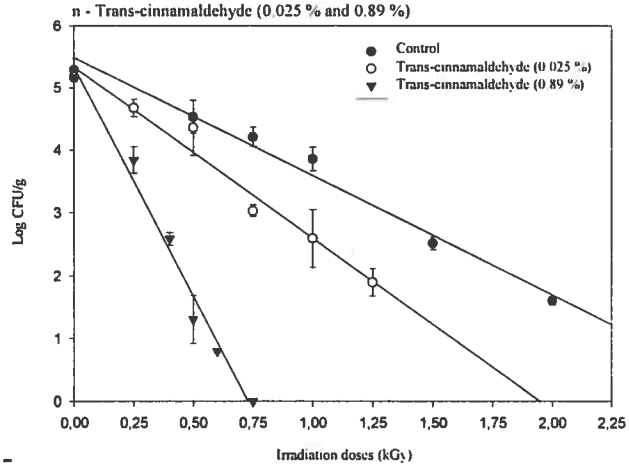
l - Thyme (0.2 % and 2.75 %)



m - Thymol (0.1 % and 1.6 %)



n - Trans-cinnamaldehyde (0.025 % and 0.89 %)



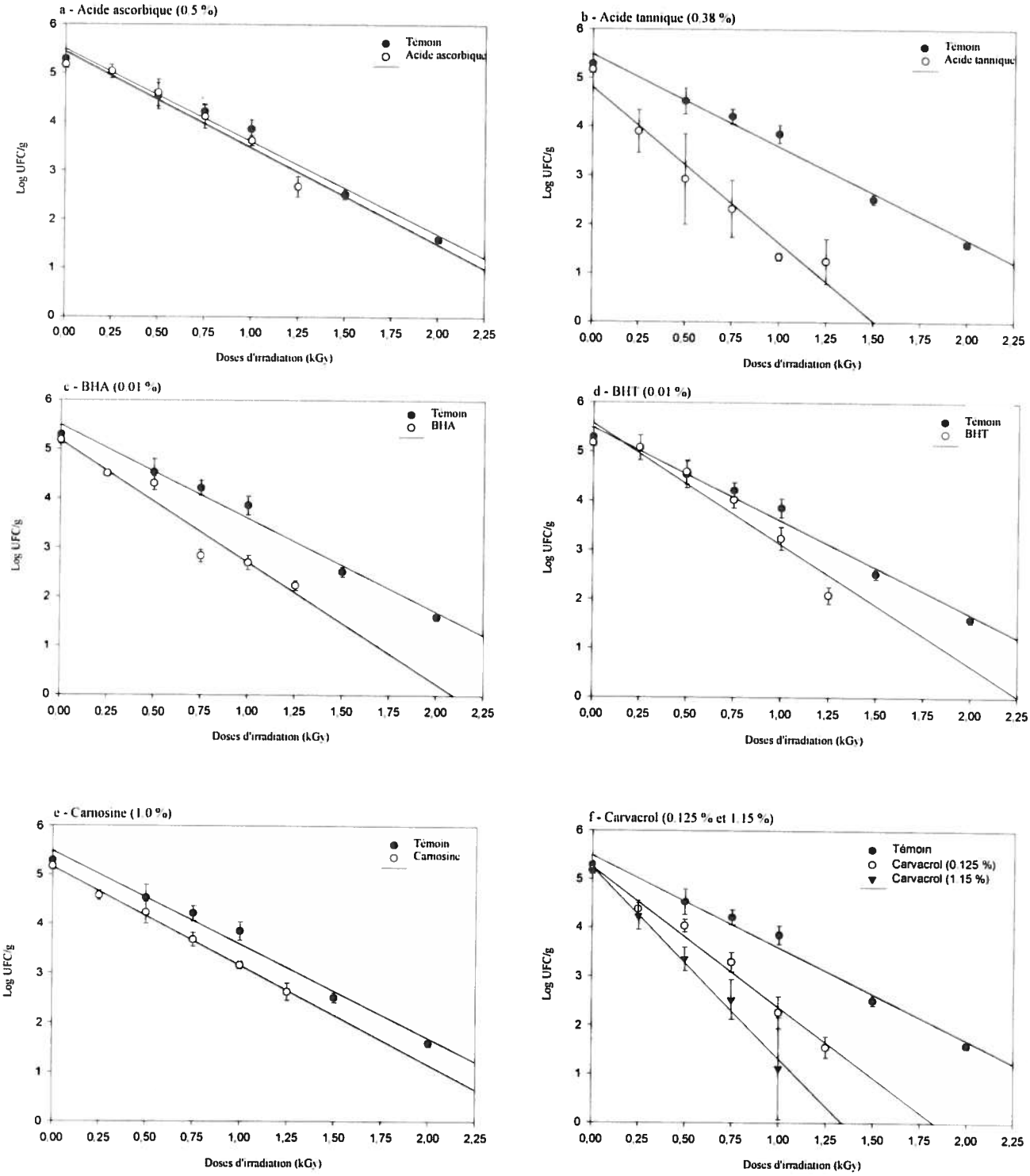
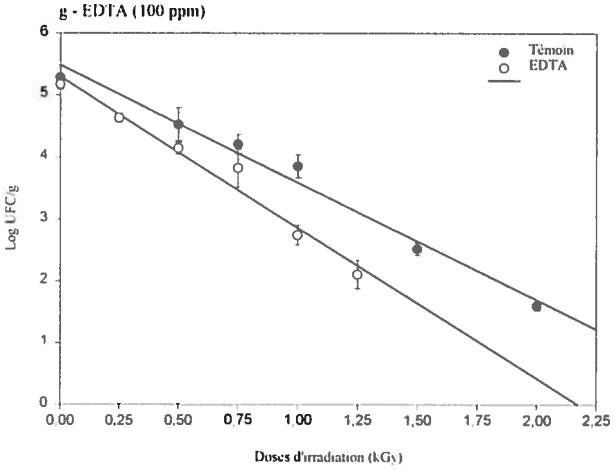
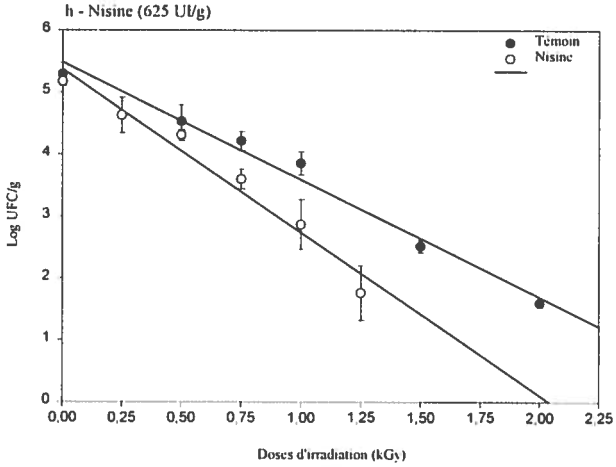


Figure 6a : Sensibilité de *S. typhi* à l'irradiation en présence de composés actifs dans du boeuf haché

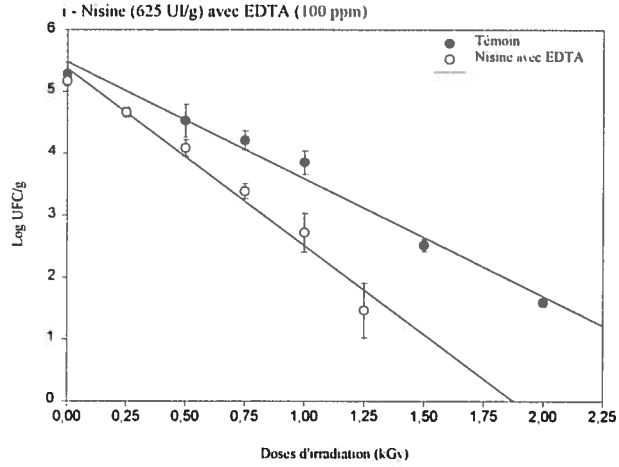
g - EDTA (100 ppm)



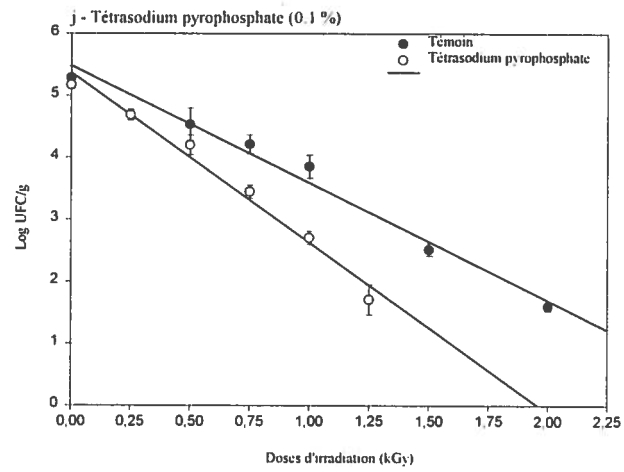
h - Nisine (625 UI/g)



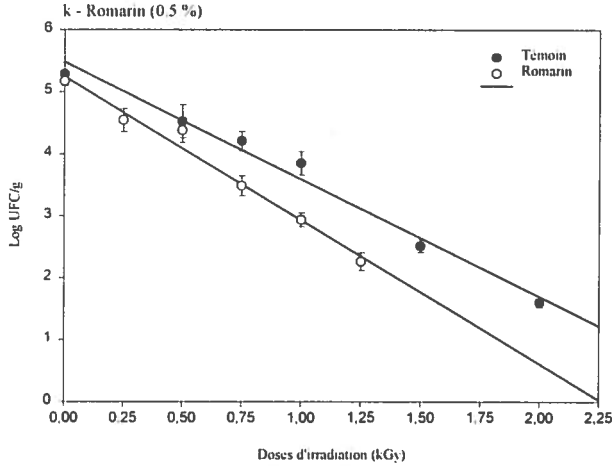
i - Nisine (625 UI/g) avec EDTA (100 ppm)



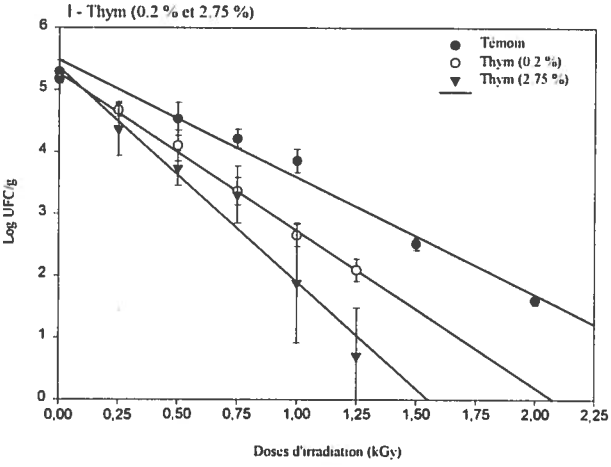
j - Tétrasodium pyrophosphate (0.1 %)



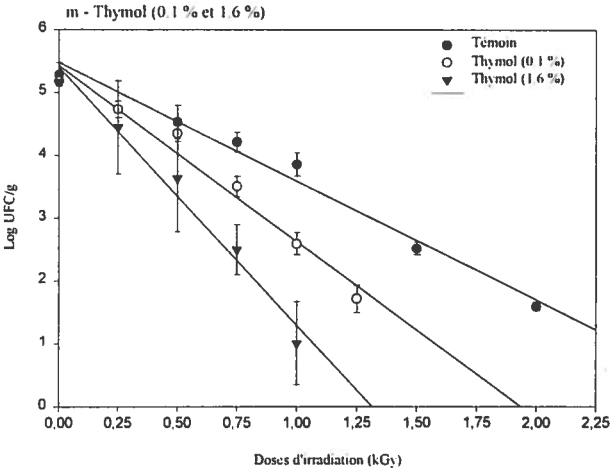
k - Romarin (0.5 %)



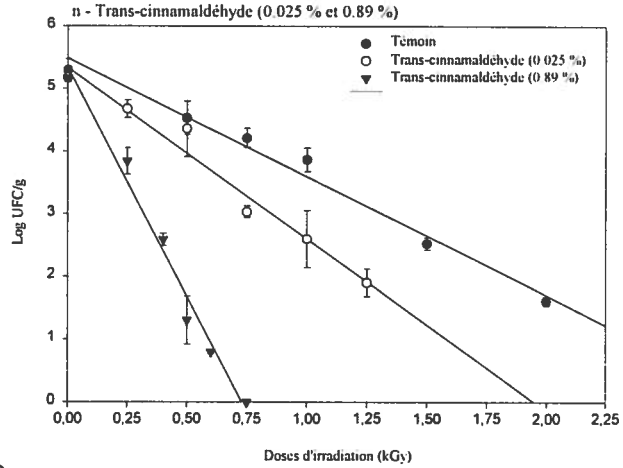
l - Thym (0.2 % et 2.75 %)



m - Thymol (0.1 % et 1.6 %)



n - Trans-cinnamaldéhyde (0.025 % et 0.89 %)



4.3. Determination of the effect of various concentrations of carvacrol on *E. coli* and *S. typhi* after irradiation (*E. coli* : irradiated at 0.25 kGy; *S. typhi* : irradiated at 0.50 kGy)

4.3.1. *Escherichia coli*

Table 6 and figure 7 show the influence of various concentrations of carvacrol (0 to 1.4 %) on the survival level of *E. coli* after irradiation at 0.25 kGy. The addition of 0.2 % of carvacrol reduced significantly ($p \leq 0.05$) the bacterial population, from 3.098 CFU/g to 2.948 CFU/g. Results also showed that the bacterial population of *E. coli* was significantly reduce ($p \leq 0.05$) as the concentration of carvacrol increased. However, no significant difference ($p > 0.05$) between the concentrations of 0.2 % and 0.4 % of carvacrol was observed, with a bacterial population of 2.948 CFU/g for both concentrations. The percentage of reduction of the log of the bacterial population at both concentrations was 4.8 %. According to Figure 7, a significant decrease ($p \leq 0.05$) in the bacterial population was observed when the concentration of carvacrol increased from 0.6 % to 0.8 %, with about 1.5 log reduction. The concentration of *E. coli* in ground beef went from 2.660 CFU/g to 1.198 CFU/g. The percentage of reduction of the log of the bacterial population increased from 14.1 % at 0.6 % of carvacrol to 61.3 % at 0.8 % of carvacrol. *E. coli* was undetectable from ground beef with a concentration of 1.2 % of carvacrol (reduction of 100 %).

4.3.2. *Salmonella typhi*

Table 7 and figure 8 show the effect of various concentrations of carvacrol (0 to 2.0 %) on the survival level of *S. typhi* after irradiation at 0.5 kGy. Without the addition of carvacrol, the level of *S. typhi* in ground beef was 4.170 CFU/g after irradiation at 0.5 kGy. The addition of carvacrol at a concentration of 0.25 % to ground beef had no significant effect ($p > 0.05$) on the irradiation sensitivity of *S. typhi*, with a bacterial population of 4.106 CFU/g. At this concentration, the percentage of reduction of the log of the bacterial population was only 1.5 %. A significant effect ($p \leq 0.05$) was observed when carvacrol was added at concentrations higher than 0.5 %. At a concentration of 0.5 % of carvacrol, the concentration of *S. typhi* was evaluated at 3.526 CFU/g in ground beef. The concentration of *S. typhi* continued to decrease significantly ($p \leq 0.05$) with the addition of 0.75 %, 1.0 %, 1.25 % and 1.5 %, with bacterial counts of 3.192 CFU/g, 2.545 CFU/g, 0.831 CFU/g and 0.264 CFU/g, respectively. The percentage of reduction of the log of the bacterial population increased from 15.4 to 93.7 % when the concentration of carvacrol in ground beef increased from 0.5 % to 1.5 %. The biggest reduction in bacterial population occurred when the concentration of carvacrol passed from 0.75 % (percentage of reduction : 39.0 %) to 1.25 % (percentage of reduction : 79.9 %). *S. typhi* was undetectable from ground beef with a concentration of 1.75 % (percentage of reduction of 100 %).

Table 6: Effect of various concentrations of carvacrol on *E. coli*, when the ground beef is irradiated at 0.25 kGy

Concentration of carvacrol (%)	Log CFU/g[1]	Percentage of reduction (%)
0	3.098 ± 0.117^{a}	
0.2	2.948 ± 0.088^{b}	4.8 %
0.4	2.948 ± 0.068^{b}	4.8 %
0.6	2.660 ± 0.037^{c}	14.1 %
0.8	1.198 ± 0.065^{d}	61.3 %
1.0	0.843 ± 0.000^{e}	72.8 %
1.2	0.000 ± 0.000^{f}	100 %
1.4	0.000 ± 0.000^{f}	100 %

[1] Duncan - ^{a,b,c,d,e,f} Values in same columns with different letters are significantly different ($p \leq 0.05$)

Tableau 6a: Effet de différentes concentrations de carvacrol sur *E. coli* quand le bœuf haché est irradié à 0,25 kGy

Concentration de carvacrol (%)	Log UFC/g[1]	Pourcentage de réduction (%)
0	$3,098 \pm 0,117^{a}$	
0,2	$2,948 \pm 0,088^{b}$	4,8 %
0,4	$2,948 \pm 0,068^{b}$	4,8 %
0,6	$2,660 \pm 0,037^{c}$	14,1 %
0,8	$1,198 \pm 0,065^{d}$	61,3 %
1,0	$0,843 \pm 0,000^{e}$	72,8 %
1,2	$0,000 \pm 0,000^{f}$	100 %
1,4	$0,000 \pm 0,000^{f}$	100 %

[1] Duncan - ^{a,b,c,d,e,f} Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0,05$)

Table 7: Effect of various concentrations of carvacrol on *S. typhi*, when the ground beef was irradiated at 0.50 kGy

Concentration of carvacrol (%)	Log CFU/g[1]	Percentage of reduction (%)
0	4.170 ± 0.084^{a}	
0.25	4.106 ± 0.091^{a}	1.5 %
0.50	3.526 ± 0.061^{b}	15.4 %
0.75	3.192 ± 0.058^{c}	23.4 %
1.00	2.545 ± 0.112^{d}	39.0 %
1.25	0.837 ± 0.000^{e}	79.9 %
1.50	0.264 ± 0.000^{f}	93.7 %
1.75	0.000 ± 0.000^{g}	100 %
2.00	0.000 ± 0.000^{g}	100 %

[1] Duncan - ^{a,b,c,d,e,f,g} Values in same columns with different letters are significantly different ($p \leq 0.05$)

Tableau 7a: Effet de différentes concentrations de carvacrol sur *S. typhi* quand le bœuf haché est irradié à 0,5 kGy

Concentration de carvacrol (%)	Log UFC/g[1]	Pourcentage de réduction (%)
0	$4,170 \pm 0,084^{a}$	
0,25	$4,106 \pm 0,091^{a}$	1,5 %
0,50	$3,526 \pm 0,061^{b}$	15,4 %
0,75	$3,192 \pm 0,058^{c}$	23,4 %
1,00	$2,545 \pm 0,112^{d}$	39,0 %
1,25	$0,837 \pm 0,000^{e}$	79,9 %
1,50	$0,264 \pm 0,000^{f}$	93,7 %
1,75	$0,000 \pm 0,000^{g}$	100 %
2,00	$0,000 \pm 0,000^{g}$	100 %

[1] Duncan - ^{a,b,c,d,e,f,g} Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0,05$)

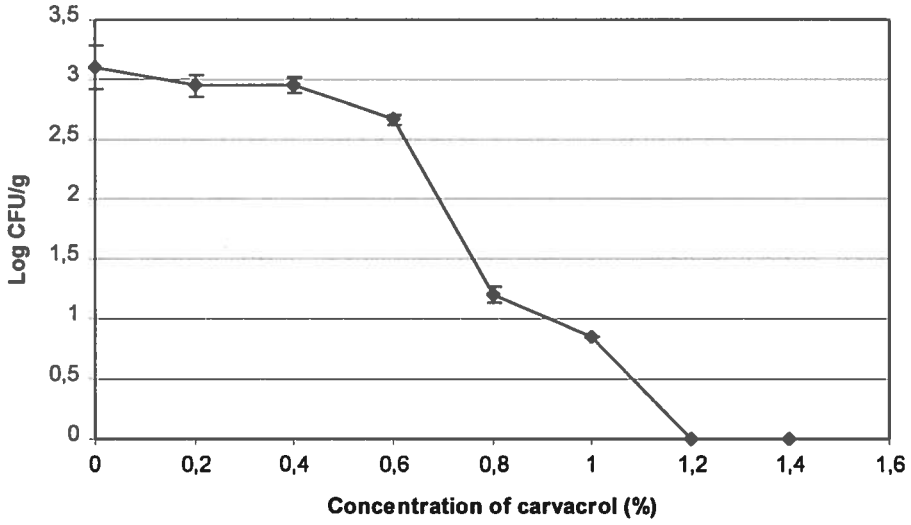


Figure 7: Effect of irradiation (0.25 kGy) along with the indicated concentrations of carvacrol on the viability of *E. coli*

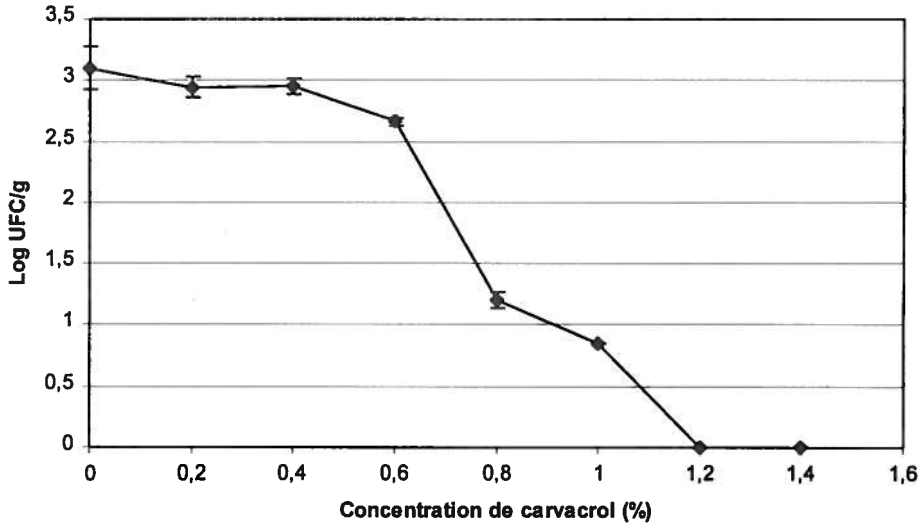


Figure 7a: Effet de différentes concentrations de carvacrol sur *E. coli*, lorsque le bœuf haché est irradié à 0,25 kGy

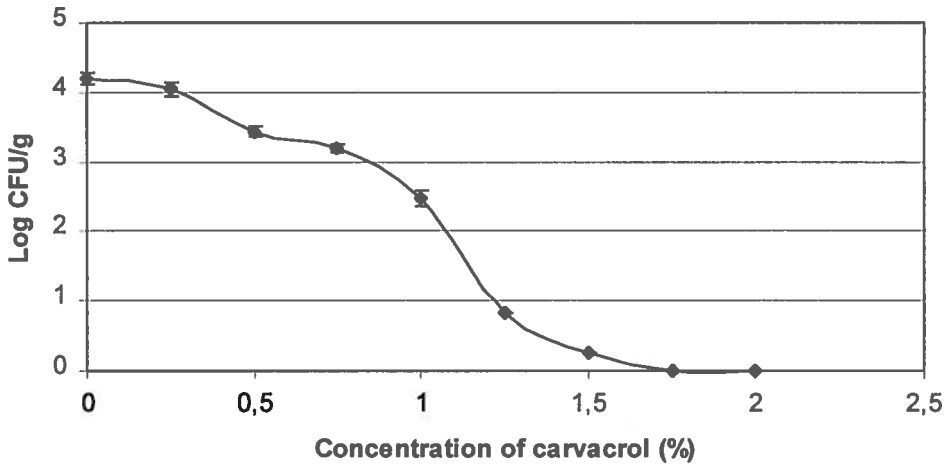


Figure 8: Effect of irradiation (0.5 kGy) along with the indicated concentrations of carvacrol on the viability of *S. typhi*

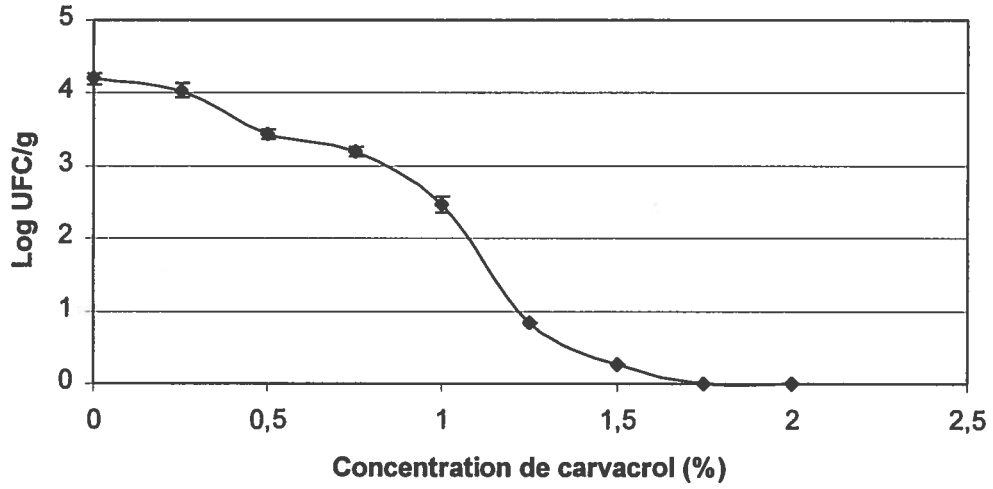


Figure 8a: Effet de différentes concentrations de carvacrol sur *S. typhi*, lorsque le bœuf haché est irradié à 0,5 kGy

4.4. Determination of the best combination of additives on the irradiation sensitivity of *E. coli* and *S. typhi*

From the result obtained for the irradiation sensitivity (D_{10}) of *E. coli* and *S. typhi* in the presence of various additives, three additives were chosen for the further experiment : carvacrol (0.88 % for *E. coli* and 1.15 % for *S. typhi*), ascorbic acid (0.5 % for both bacteria) and tetrasodium pyrophosphate (0.1 % for both bacteria). Carvacrol was chosen for its effect on the irradiation sensitivity of both bacteria, ascorbic acid for its ability to maintain the colour of the meat during irradiation and tetrasodium pyrophosphate for its water retention properties and its ability to preserve the taste of the meat after irradiation. Since the concentration used for carvacrol was different for both bacteria, only one concentration , 1.0 %, was chosen.

4.4.1. *Escherichia coli*

Table 8 and figure 9 show the irradiation sensitivity of *E. coli* in ground beef in the presence of various combination of additives. The D_{10} of the control, *E. coli* was 0.126 kGy. Of the combination tested, carvacrol and the mixture of carvacrol and tetrasodium pyrophosphate were the most efficient. The additives significantly reduced ($p \leq 0.05$) the D_{10} value from 0.126 kGy without additives to 0.057 kGy in the presence of carvacrol (1.0 %) and in the presence of the mixture of carvacrol (1.0 %) and tetrasodium pyrophosphate (0.1 %), meaning an increase of irradiation sensitivity by 55.5 %. These two combinations were the only ones that increased the irradiation sensitivity of *E. coli* in this test. The addition of the mixture of carvacrol and ascorbic acid had no significant effect ($p > 0.05$) on the irradiation sensitivity of *E. coli*. The D_{10} was 0.133 kGy. The addition of the mixture of carvacrol, ascorbic acid and tetrasodium pyrophosphate significantly increased ($p \leq 0.05$) the D_{10} value to 0.142 kGy, meaning a protective effect of these additives on *E. coli* by a percentage of 10.9 %.

According to figure 9, *E. coli* without additives was undetectable at an irradiation dose of 0.7 kGy. In the presence of the mixture of carvacrol and ascorbic acid, a dose of 0.7 kGy was also necessary to reduce *E. coli* to an undetectable level from ground beef. When carvacrol and the mixture of carvacrol and tetrasodium pyrophosphate were added to the ground beef, the irradiation dose necessary to reduce *E. coli* under the detection level was reduced to 0.3 kGy. It was, however increased to 0.75 kGy, when the mixture of carvacrol, ascorbic acid and tetrasodium pyrophosphate was added to the ground beef.

This experiment demonstrated that the addition of carvacrol and the mixture of carvacrol and tetrasodium pyrophosphate were the most efficient. The irradiation sensitivity of *E. coli* was increased by 55.5 %. The irradiation dose necessary to eliminate completely *E. coli* in the presence of those additives was 2.3 times lower than in the absence of additives.

4.4.2. *Salmonella typhi*

Table 9 and figure 10 show the irradiation sensitivity of *S. typhi* in ground beef in the presence of various combination of additives. The D_{10} of *S. typhi* was 0.526 kGy. All of the combination tested significantly increased ($p \leq 0.05$) the irradiation sensitivity of *S. typhi*. The most efficient combination was carvacrol and the mixture of carvacrol and tetrasodium pyrophosphate, with D_{10} values of 0.235 kGy and 0.254 kGy respectively. Their was no significant difference ($p > 0.05$) between these two combinations. The mixture of carvacrol, ascorbic acid and tetrasodium pyrophosphate was the third most effective combination, with a D_{10} value of 0.313 kGy, followed by the mixture of carvacrol and ascorbic acid, with a D_{10} of 0.344 kGy. The efficiency of those combination ranged from 54.7 % to 33.7 %.

According to figure 10, a presence of $10^{1.2}$ CFU/g was observed when samples without additives were treated to 2.25 kGy. When carvacrol and the mixture of carvacrol and tetrasodium pyrophosphate were added to the ground beef, *S. typhi* was undetectable at a doses of 1.25 kGy and 1.0 kGy respectively. With the addition of the mixture of carvacrol and ascorbic acid and the mixture of carvacrol, ascorbic acid and tetrasodium pyrophosphate, *S. typhi* was undetectable in ground beef with an irradiation dose of 1.5 kGy and 1.7 kGy respectively. These results suggest that the addition of the best combination of additives, the mixture of carvacrol and tetrasodium pyrophosphate in ground beef was able to reduce by 2.5 times the irradiation dose necessary to reduce *S. typhi* under the detection level.

Table 8: Irradiation sensitivity (D_{10}) of *E. coli* in presence of carvacrol (1.0 %) alone or in combination with ascorbic acid (0.5%) and tetrasodium pyrophosphate (0.1%)

Additives	D_{10} (kGy)[1]	Efficiency[2]
Control	0.126 ± 0.0036^{b}	
carvacrol (1.0 %)	0.057 ± 0.0015^{a}	55.5 %
carvacrol (1.0 %) with tetrasodium pyrophosphate (0.1%)	0.057 ± 0.0010^{a}	55.5 %
carvacrol (1.0%) with ascorbic acid (0.5%)	0.133 ± 0.0043^{b}	- 3.9 %
carvacrol (1.0 %) with ascorbic acid (0.5%) and tetrasodium pyrophosphate (0.1%)	0.142 ± 0.0051^{c}	- 10.9 %

[1] Duncan– [abc] Values in same columns with different letters are significantly different ($p \leq 0.05$)
[2] A "–" sign before a number represent a protective effect on *E. coli*

Tableau 8a: Sensibilité à l'irradiation (D_{10}) de *E. coli* en présence de carvacrol (1,0 %) seul ou en combinaison avec de l'acide ascorbique (0,5%) et du tétrasodium pyrophosphate (0,1%)

Additifs	D_{10} (kGy)[1]	Efficacité[2]
Témoin	$0,126 \pm 0,0036^{b}$	
carvacrol (1,0 %)	$0,057 \pm 0,0015^{a}$	55,5 %
carvacrol (1.0 %) avec tétrasodium pyrophosphate (0,1%)	$0,057 \pm 0,0010^{a}$	55,5 %
carvacrol (1,0%) avec acide ascorbique (0,5%)	$0,133 \pm 0,0043^{b}$	- 3,9 %
carvacrol (1.0 %) avec acide ascorbique (0,5%) et tétrasodium pyrophosphate (0,1%)	$0,142 \pm 0,0051^{c}$	- 10,9 %

[1] Duncan– [abc] Valeurs dans la même colonne avec une lettre différentes sont significativement différent ($p \leq 0,05$)
[2] Les valeurs avec un " - " ont un effet protecteur sur la bactérie comparativement au témoin

Table 9: Irradiation sensitivity (D_{10}) of *S. typhi* in presence of carvacrol (1.0 %) alone or in combination with ascorbic acid (0.5%) and tetrasodium pyrophosphate (0.1%)

Additives	D_{10} (kGy)[1]	Efficiency
Control	0.526 ± 0.0161^{d}	
carvacrol (1.0 %)	0.235 ± 0.0158^{a}	54.7 %
carvacrol (1.0 %) with tetrasodium pyrophosphate (0.1%)	0.254 ± 0.0102^{a}	51.0 %
carvacrol (1.0 %) with ascorbic acid (0.5%) and tetrasodium pyrophosphate (0.1%)	0.313 ± 0.0085^{b}	39.7 %
carvacrol (1.0%) with ascorbic acid (0.5%)	0.344 ± 0.0086^{c}	33.7 %

[1] LSD and Duncan– [abc] Values in same columns with different letters are significantly different ($p \leq 0.05$)

Tableau 9a: Sensibilité à l'irradiation (D_{10}) de *S. typhi* en présence de carvacrol (1,0 %) seul ou en combinaison avec de l'acide ascorbique (0,5%) et du tétrasodium pyrophosphate (0,1%)

Additifs	D_{10} (kGy)[1]	Efficacité
Control	0.526 ± 0.0161^{d}	
Carvacrol (1,0 %)	$0,235 \pm 0,0158^{a}$	54,7 %
Carvacrol (1.0 %) avec tétrasodium pyrophosphate (0,1%)	$0,254 \pm 0,0102^{a}$	51,0 %
Carvacrol (1.0 %) avec acide ascorbique (0,5%) et tétrasodium pyrophosphate (0,1%)	$0,313 \pm 0,0085^{b}$	39,7 %
Carvacrol (1,0%) avec acide ascorbique (0,5%)	$0,344 \pm 0,0086^{c}$	33,7 %

[1] Duncan– [abc] Valeurs dans la même colonne avec une lettre différentes sont significativement différent ($p \leq 0,05$)

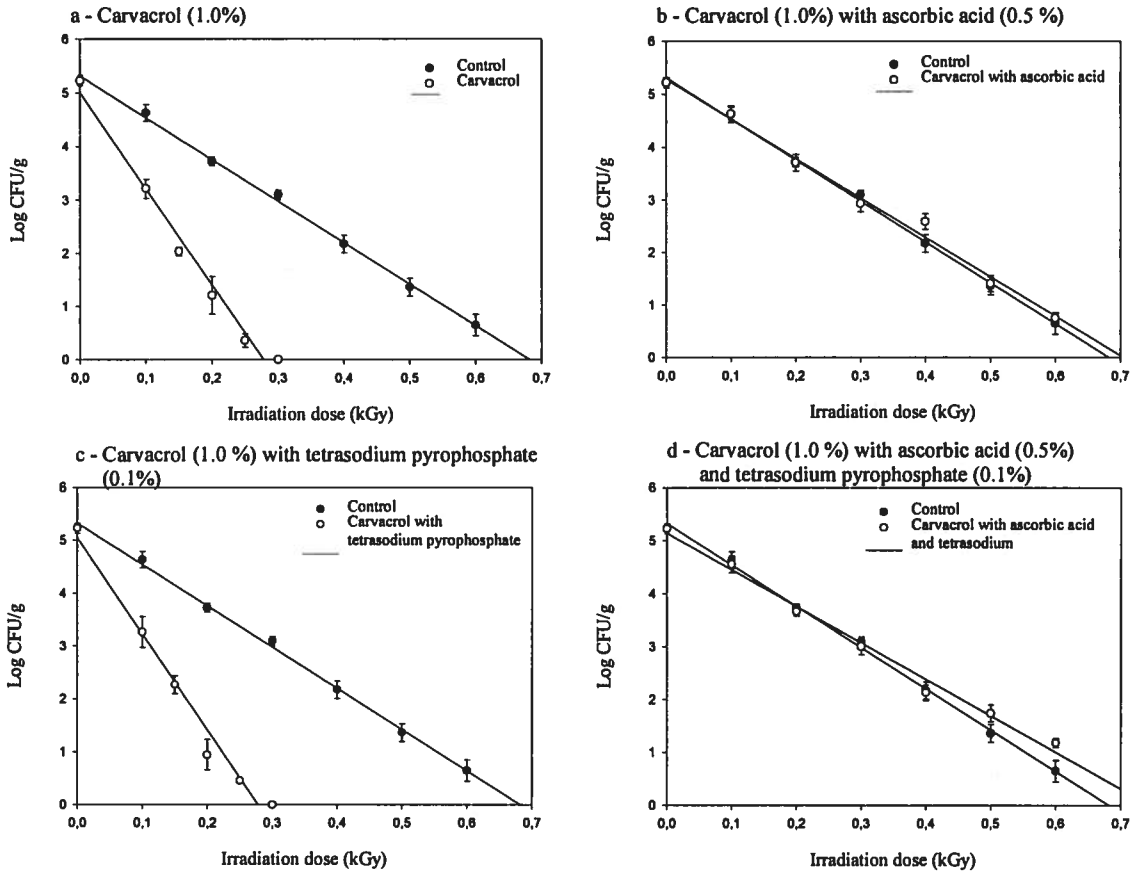


Figure 9 : Irradiation sensitivity of *E. coli* in presence of carvacrol (1.0%),carvacrol (1.0%) with ascorbic acid (0.5%), carvacrol (1.0%) with tetrasodium pyrophosphate (0.1%) and carvacrol (1.0%) with ascorbic acid (0.5%) and tetrasodium pyrophosphate (0.1%)

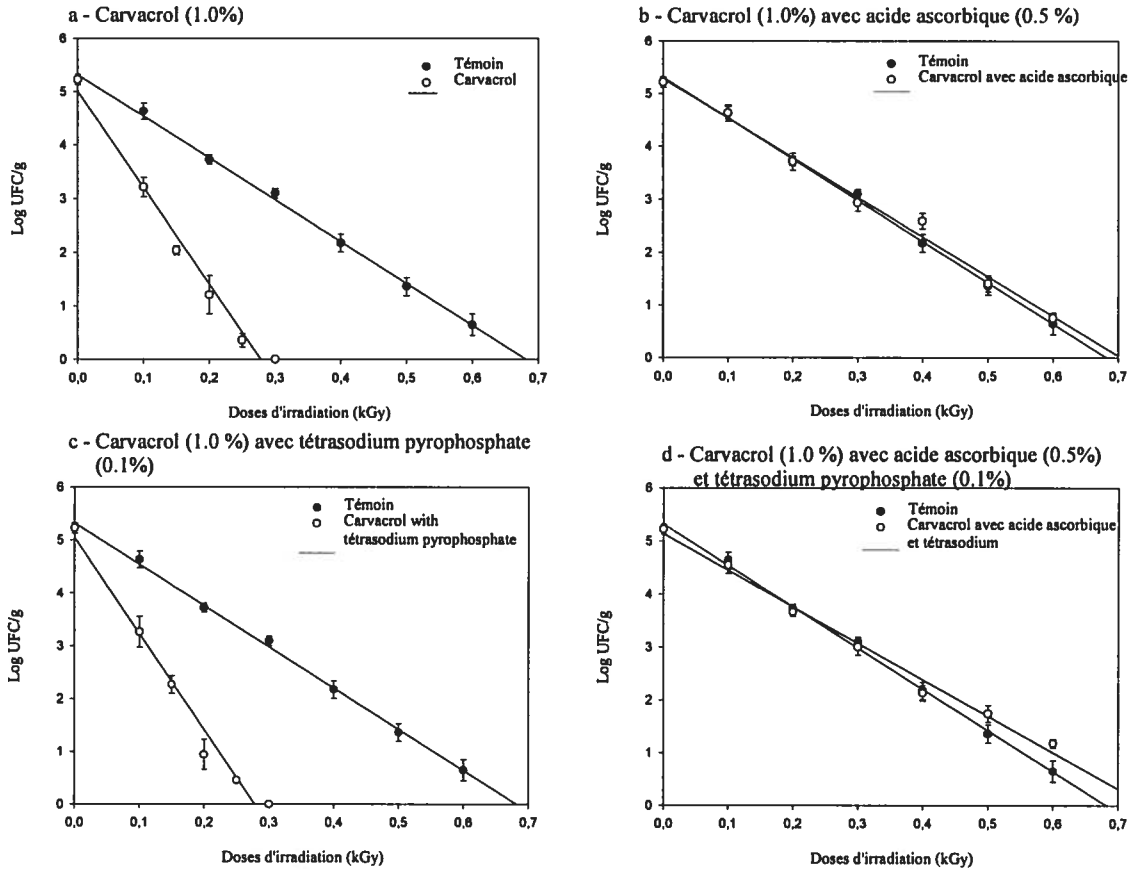


Figure 9a : Sensibilité de *E. coli* à l'irradiation en présence de carvacrol (1.0%),carvacrol (1.0%) avec acide ascorbique(0.5%), carvacrol (1.0%) avec tétrasodium pyrophosphate (0.1%) et carvacrol (1.0%) avec acide ascorbique (0.5%) et tétrasodium pyrophosphate (0.1%)

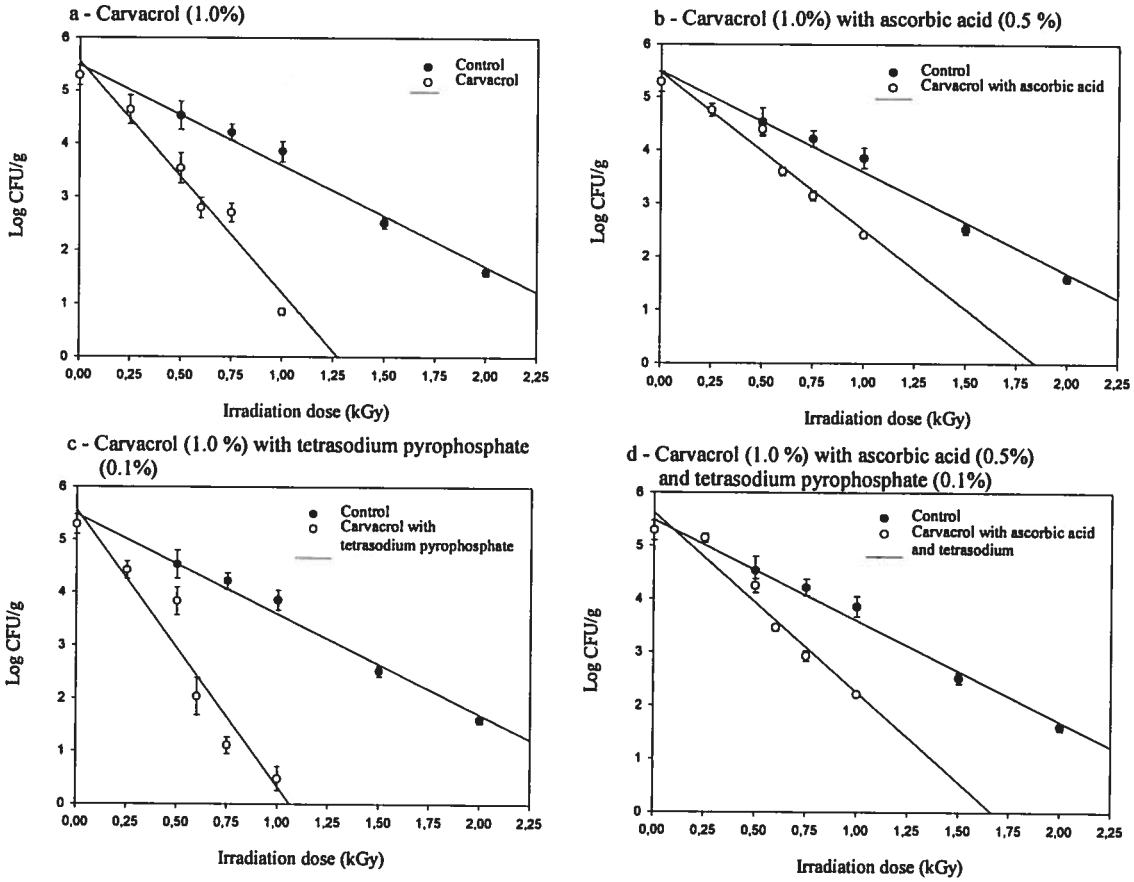


Figure 10: Irradiation sensitivity of *S. typhi* in the presence of carvacrol (1.0%), carvacrol (1.0%) with ascorbic acid (0.5%), carvacrol (1.0%) with tetrasodium pyrophosphate (0.1%) and carvacrol (1.0%) with ascorbic acid (0.5%) and tetrasodium pyrophosphate (0.1 %)

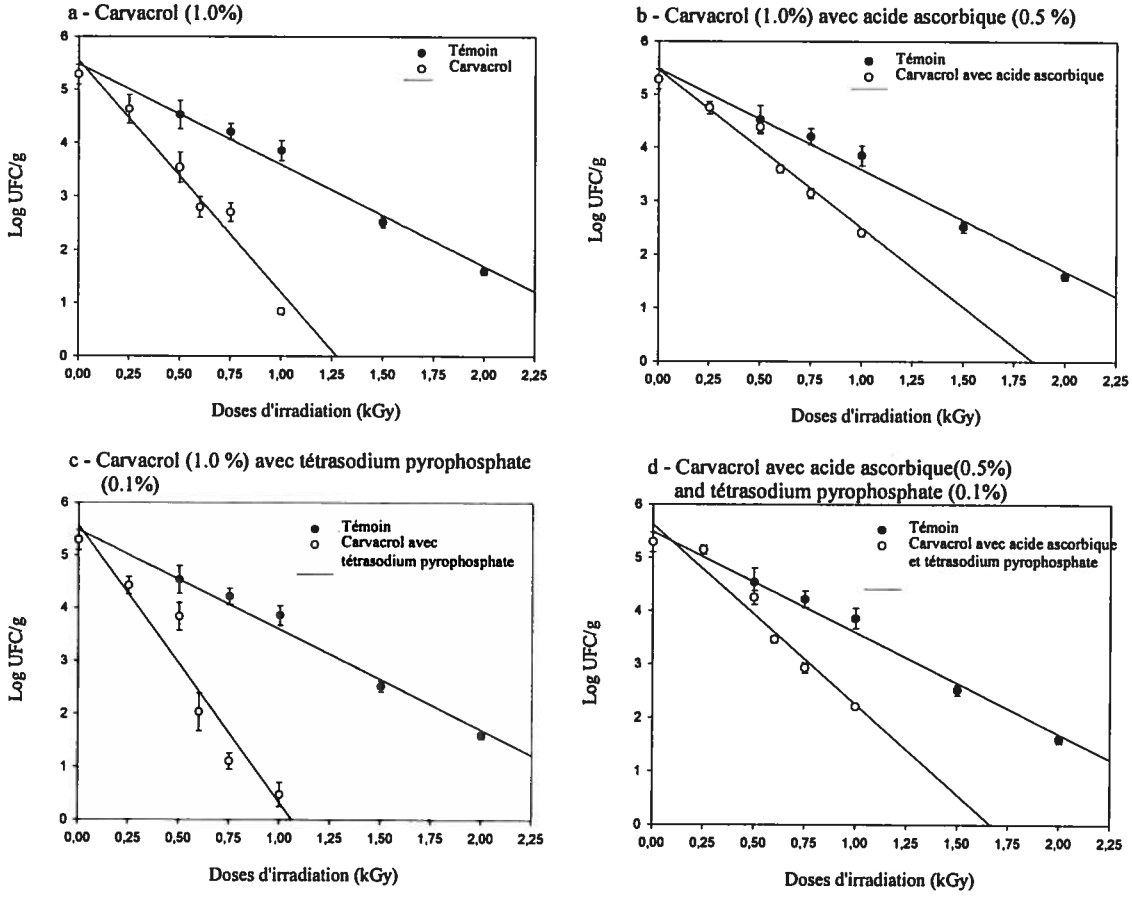


Figure 10a: Sensibilité de *S. typhi* en présence de carvacrol (1.0 %), carvacrol (1.0%) et acide ascorbique (0.5%), carvacrol (1.0%) avec tétrasodium pyrophosphate (0.1%) et carvacrol (1.0%) avec acide ascorbique (0.5%) et tétrasodium pyrophosphate (0.1 %)

4.5. Effect of headspace atmosphere on the irradiation sensitivity of *E. coli* and *S. typhi*

4.5.1. *Escherichia coli*

Tables 10 and 11 and figure 11 show the irradiation sensitivity (D_{10}) of *E. coli* in ground beef under various atmospheres, air, CO_2 and MAP and vacuum packaging. In general, the addition of the additives to the samples increased the irradiation sensitivity of *E. coli*, regardless of the headspace atmosphere present during irradiation. According to these results, MAP had the greatest inhibitory effect on *E. coli* with a D_{10} of 0.086 kGy, which was significantly different from all the other atmospheres tested ($p \leq 0.05$). MAP increased the irradiation sensitivity of *E. coli* by 37.7 %. When carvacrol and tetrasodium pyrophosphate were added to the ground beef packed under MAP, the D_{10} value was 0.046 kGy, which represents an efficiency of 46.5 %. The irradiation sensitivity was 16.4 % more than samples packed under air and in the presence of additives, with a D_{10} of 0.055 kGy.

When ground beef was packed under CO_2 atmosphere, the D_{10} observed was 0.123 kGy. No significant difference ($p > 0.05$) was observed in irradiation sensitivity of the ground beef packed under CO_2 and under air, where the D_{10} values were evaluated at 0.123 kGy and 0.126 kGy, respectively. No significant difference ($p > 0.05$) was also observed in irradiation sensitivity of the ground beef packed under CO_2 and under vacuum, where the D_{10} values were evaluated at 0.123 kGy and 0.118 kGy, respectively. Under CO_2 atmosphere, the influence of the atmosphere was evaluated at only 2.4 %. When carvacrol and tetrasodium pyrophosphate were added in samples treated under CO_2, there was an increase in the irradiation sensitivity. The D_{10} value decreased from 0.123 kGy to 0.106 kGy, representing an efficiency of 13.8 %.

When samples were packed under air, the D_{10} value of *E. coli* was 0.126 kGy. However, when carvacrol and tetrasodium pyrophosphate are present in the ground beef, a significant increase in the irradiation sensitivity ($p \leq 0.05$) of *E. coli* was observed, representing a D_{10} value of 0.055 kGy. The efficiency of the additives was 56.3 % under air.

Under vacuum conditions, the D_{10} value of *E. coli* was 0.118 kGy. A significant increase in irradiation sensitivity ($p \leq 0.05$) was observed compared to air packed ground beef, where the D_{10} value was 0.126 kGy, representing an efficiency of 6.3 %. The D_{10} value was significantly lower ($p \leq 0.05$) with the addition of carvacrol and tetrasodium pyrophosphate, which was evaluated at 0.101 kGy, representing an efficiency of 14.4 %. In presence of the additives, *E. coli* was more resistant under vacuum than under air condition, with a decrease in irradiation sensitivity by 83.6 %.

The combination of additives and packaging atmosphere affected the irradiation dose necessary to reduce *E. coli* under the detection level in ground beef. Under air, this dose was 0.7 kGy without the presence of additives and 0.3 kGy when additives were added. When ground beef was packed under the best atmosphere, which was MAP, the dose was reduce to 0.45 kGy without additives and to 0.25 kGy with additives. These values represent a reduction in dose by 1.5 times and 1.2 times compared to those under air. Under vacuum, the reduction was not as great as with MAP without additives. When additives were added, there was an increase in the dose necessary to reduce *E. coli* under the detection level. They were 0.65 kGy without additives and 0.55 kGy with additives, meaning a reduction by 1.08 times and an increase by 1.8 times. Finally, under CO_2, *E. coli* was undetectable at 0.7 kGy, identical as under air. When additives were added, the irradiation dose necessary doubled compared to under air. It went from 0.3 kGy for under air to 0.6 kGy for under CO_2.

Without additives, the best treatment was under MAP, representing an efficiency of 37.7 %, followed by vacuum, with an efficiency of 6.3 % and by CO_2, with an efficiency of 2.4 %, compared to the air treatment. In the presence of the additives, the best treatment was MAP, representing an efficiency of 16.4 %. A protective effect was observed under vacuum and CO_2, with protective effect of 83.6 % and 92.7 %, respectively. This means that the vacuum and CO_2 helps to protect *E. coli* when carvacrol and tetrasodium pyrophosphate are present.

4.5.2. *Salmonella typhi*

Tables 12 and 13 and figure 12 show the irradiation sensitivity (D_{10}) of *S. typhi* in ground beef under various atmospheres : air; CO_2; MAP and vacuum packaging. The most important inhibitory effect during irradiation was observed under MAP. The D_{10} value of *S. typhi* was 0.221 kGy, which was significantly lower ($p \leq 0.05$) than ground beef packed under air (0.526 kGy), under CO_2 (0.420 kGy) and under vacuum (0.429 kGy). MAP helped to increase in irradiation sensitivity of *S. typhi* by 58.0 % compared to the air packed ground beef. In the presence of carvacrol and tetrasodium pyrophosphate and packed under MAP, the D_{10} value was reduced to 0.053 kGy, which represent an efficiency of the additives of 76.0 %. The combination of the additives with the MAP increased the irradiation sensitivity by 79.1 %.

When packed under air conditions, the D_{10} value was 0.526 kGy and this value was significantly higher ($p \leq 0.05$) than all the other atmospheres tested for *S. typhi*. When carvacrol and tetrasodium pyrophosphate were added in the ground beef, the sensitivity of *S. typhi* increases showing a D_{10} of 0.254 kGy. This represents an efficiency of 51.7 %.

When ground beef was packed under CO_2 atmosphere, the D_{10} value observed for *S. typhi* was 0.420 kGy. This value was similar to the value obtained for vacuum packaging. As compared to air condition, the CO_2 atmosphere resulted in an increase of irradiation sensitivity by 18.4 % (0.526 kGy vs 0.420 kGy). When carvacrol and tetrasodium pyrophosphate were added under 100 % CO_2, an increase of the irradiation sensitivity was observed, with a D_{10} value of 0.336 kGy. Compared with the ground beef packed under air and in presence of additives, there was a decrease of 32.3 % in irradiation sensitivity. This means that the CO_2 helps to protect *S. typhi* in the presence of carvacrol and tetrasodium pyrophosphate. Even with this protective effect, this additives had an effect on the irradiation sensitivity of *S. typhi* with an efficiency of 20.0 %.

Under vacuum, the D_{10} value of *S. typhi* observed was 0.429 kGy compared to 0.526 kGy under air condition. The vacuum atmosphere increased the irradiation sensitivity by 18.4 % compared to air atmosphere conditions. The D_{10} value of *S. typhi* in the presence of carvacrol and tetrasodium pyrophosphate and packed under vacuum was 0.308 kGy, and this was significantly lower ($p \leq 0.05$) than the D_{10} value of *S. typhi* without any additives. The efficiency of this treatment to eliminate *S, typhi* was 28.2 %. In the presence of carvacrol and tetrasodium pyrophosphate, *S. typhi* was 21.2 % more resistant to irradiation under vacuum than under air in the presence of the same additives. The D_{10} value were respectively 0.308 kGy and 0.254 kGy. Vacuum packaging seams to protect *S. typhi* during the irradiation treatment.

The results obtained in this experiment showed that the best irradiation sensibility of *S. typhi* was under MAP condition, with a D_{10} value of 0.221 kGy compared to 0.526 kGy under air, representing an efficiency of 58.0 %. This was followed by CO_2 atmosphere (0.420 kGy), with an efficiency of 20.2 %, vacuum (0.429 kGy), with an efficiency of 18.4 % and finally with the control, the air packed samples (0.526 kGy).

The combination of additives and packaging atmosphere affected the irradiation dose necessary to reduce *S. typhi* under the detection level in ground beef (figure 12). Under air, at an irradiation dose of 2.0 kGy, 1.5 log of bacteria remained in the ground beef. With the addition of additives, a dose of 1.3 kGy was needed to reduce the bacteria under the detection level. When ground beef was packed under the best atmosphere, which was MAP, the dose was 1.55 kGy without additives and to 0.25 kGy with additives. In the presence of additives, this value represented a reduction in dose by 5.2 times compared to that under air with additives. Under CO_2 and under vacuum, the reduction was not as great as with MAP with or without additives. Without additives, a concentration of 1 log of bacteria was still present in the ground beef after an irradiation treatment of 2 kGy. When the additives were added, the irradiation dose necessary to reduce *S. typhi* under the detection level went from 1.3 kGy under air to 1.8 kGy under CO_2 and to 1.6 kGy under vacuum. These doses represents an increase by 1.4 times and 1.2 times respectively.

In the presence of the additives, the best condition to eliminate *S. typhi* was also under MAP with a D_{10} value of 0.053 kGy, compared to under air with a D_{10} of 0.254 kGy. The influence of the atmosphere was evaluated at 79.1 %. Ground beef in the presence of additives under air was second, with a D_{10} value of 0.254 kGy. When ground beef was packed under vacuum or under CO_2, a protective effect of 83.6 % and 92.7 % respectively was observed compared to ground beef under air. The D_{10} value were 0.308 kGy and 0.336 kGy.

Table 14 show the results of the variance analysis on the significance of simple and combined effect of the addition of the mixture of additives (carvacrol with tetrasodium pyrophosphate) with packaging conditions on the irradiation sensitivity of *E. coli* and *S. typhi*. According to the results, the addition of additives and the packaging atmosphere had a significant effect ($p \leq 0.001$) on the irradiation sensitivity of *E. coli* and *S. typhi*. An interaction ($p \leq 0.001$) was observed between the addition of the additives and the packaging atmosphere on the irradiation sensitivity of *E. coli* and *S. typhi*. These results suggest that there was a synergistic effect between the addition of additives and the various packaging atmosphere tested in this study on the irradiation sensitivity of *E. coli* and *S. typhi*.

Table 10 : Irradiation sensitivity of *E. coli* in ground beef as affected by a mixture of carvacrol (1 %) and tetrasodium pyrophosphate (0.1 %) under various atmospheres

Packaging atmosphere	D_{10} (kGy)[1,2]		Efficiency (%) of the additives
	Control	Carvacrol(1%) and tetrasodium pyrophosphate(0.1%)	
MAP[3]	0.086 ± 0.0030^{a}	$0.046 \pm 0.0008^{a*}$	46.5 %
Vacuum	0.118 ± 0.0054^{b}	$0.101 \pm 0.0036^{c*}$	14.4 %
100 % CO_2	0.123 ± 0.0068^{bc}	$0.106 \pm 0.0048^{d*}$	13.8 %
Air	0.126 ± 0.0036^{c}	$0.055 \pm 0.0014^{b*}$	56.3 %

[1] Duncan– [abcd] Values in same columns with different letters are significantly different ($p \leq 0.05$)

[2] T-Test - Values in same rows with a " * " are significantly different ($p \leq 0.05$)

[3] 60% O_2–30% CO_2-10% N_2

Tableau 10a : Sensibilité l'irradiation de *E. coli* dans du bœuf haché affecté par un mélange de carvacrol (1 %) et tétrasodium pyrophosphate (0,1 %) sous différents atmosphères

Atmosphère d'emballage	D_{10} (kGy)[1,2]		Efficacité des additifs
	Témoin	Carvacrol (1%) et tétrasodium pyrophosphate (0,1%)	
MAP[3]	$0,086 \pm 0,0030^{a}$	$0,046 \pm 0,0008^{a*}$	46,5 %
Sous vide	$0,118 \pm 0,0054^{b}$	$0,101 \pm 0,0036^{c*}$	14,4 %
100 % CO_2	$0,123 \pm 0,0068^{bc}$	$0,106 \pm 0,0048^{d*}$	13,8 %
Air	$0,126 \pm 0,0036^{c}$	$0,055 \pm 0,0014^{b*}$	56,3 %

[1] Duncan– [abcd] Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0,05$)

[2] T-Test – Valeurs dans une même rangé avec un " * " sont significativement différentes ($p \leq 0,05$)

[3] 60% O_2 – 30% CO_2 - 10% N_2

Table 11 : Percentage of efficiency of the different modified atmosphere on the irradiation sensitivity of *E. coli* when compared to air packaging

Packaging atmosphere	Percentage of efficiency[2]	
	Control	Carvacrol (1%) and tetrasodium pyrophosphate (0.1 %)
MAP[1]	37.7 %	16.4 %
Vacuum	6.3 %	- 83.6 %
100 % CO_2	2.4 %	- 92.7 %

[1] 60% O_2–30% CO_2-10% N_2

[2] A negative value represent a protective effect on the bacteria

Tableau 11a : Pourcentage d'efficacité des différents atmosphères modifiés sur la sensibilité de *E. coli* en comparaison avec l'emballage sous air

Atmosphère d'emballage	Pourcentage d'efficacité[2]	
	Témoin	Carvacrol (1%) et tétrasodium pyrophosphate (0,1 %)
MAP[1]	37,7 %	16,4 %
Sous - vide	6,3 %	- 83,6 %
100 % CO_2	2,4 %	- 92,7 %

[1] 60% O_2–30% CO_2-10% N_2

[2] Une valeur négative représente un effet protecteur sur la bactérie

Table 12 : Irradiation sensitivity of *S. typhi* in ground beef as affected by a mixture of carvacrol (1 %) and tetrasodium pyrophosphate (0.1 %) under various atmospheres

Packaging atmosphere	D_{10} (kGy)[12]		Efficiency (%) of the additives
	Control	Carvacrol(1%) with tetrasodium pyrophosphate(0.1%)	
MAP[3]	0.221 ± 0.0189^{a}	$0.053 \pm 0.0012^{a*}$	76.0 %
100 % CO_2	0.420 ± 0.0046^{b}	$0.336 \pm 0.0280^{d*}$	20.0 %
Vacuum	0.429 ± 0.0089^{b}	$0.308 \pm 0.0132^{c*}$	28.2 %
Air	0.526 ± 0.0161^{c}	$0.254 \pm 0.0102^{b*}$	51.7 %

[1] Duncan– [abcd] Values in same columns with different letters are significantly different ($p \leq 0.05$)

[2] T-Test - Values in same rows with a " * " are significantly different ($p \leq 0.05$)

[3] 60% O_2–30% CO_2-10% N_2

Tableau 12a : Sensibilité l'irradiation de *S. typhi* dans du bœuf haché affecté par un mélange de carvacrol (1 %) et tétrasodium pyrophosphate (0,1 %) sous différents atmosphères

Atmosphère d'emballage	D_{10} (kGy)[12]		Efficacité (%) des additifs
	Témoin	Carvacrol(1%) et tétrasodium pyrophosphate(0,1%)	
MAP[3]	$0,221 \pm 0,0189^{a}$	$0,053 \pm 0,0012^{a*}$	76,0 %
100 % CO_2	$0,420 \pm 0,0046^{b}$	$0,336 \pm 0,0280^{d*}$	20,0 %
Sous - vide	$0,429 \pm 0,0089^{b}$	$0,308 \pm 0,0132^{c*}$	28,2 %
Air	$0,526 \pm 0,0161^{c}$	$0,254 \pm 0,0102^{b*}$	51,7 %

[1] Duncan– [abcd] Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0,05$)

[2] T-Test – Valeurs dans une même rangé avec un " * " sont significativement différentes ($p \leq 0,05$)

[3] 60% O_2–30% CO_2-10% N_2

Table 13 : Percentage of efficiency (compared to air packaging) of the different modified atmosphere tested on the irradiation sensitivity of *S. typhi*

Packaging atmosphere	Percentage of efficiency[2]	
	Control	Carvacrol (1%) with tetrasodium pyrophosphate (0.1 %)
MAP[1]	58.0 %	79.1 %
100 % CO_2	20.2 %	- 32.3 %
Vacuum	18.4 %	- 21.2 %

[1] 60% O_2–30% CO_2-10% N_2

[2] A negative value represent a protective effect on the bacteria

Tableau 13a : Pourcentage d'efficacité des différents atmosphères modifiés sur la sensibilité de *S. typhi* en comparaison avec l'emballage sous air

Atmosphère d'emballage	Pourcentage d'efficacité[2]	
	Témoin	Carvacrol (1%) et tétrasodium pyrophosphate (0,1 %)
MAP[1]	58,0 %	79,1 %
100 % CO_2	20,2 %	- 32,3 %
Sous - vide	18,4 %	- 21,2 %

[1] 60% O_2–30% CO_2-10% N_2

[2] Une valeur négative représente un effet protecteur sur la bactérie

Table 14 : Results of variance analysis showing the significance of simple and combined effects of the addition of the mixture of additives (carvacrol with tetrasodium pyrophosphate) and the packaging atmosphere on the irradiation sensitivity of *E. coli* and *S. typhi* in ground beef

Factors	DF[1]	P (F > Fcal)[2]	
		E. coli	*S. typhi*
Additives	1	< 0.001	< 0.001
Atmosphere	3	< 0.001	< 0.001
Additives * atmosphere	3	< 0.001	< 0.001

[1]Degree of freedom

[2]Simple and combined effects are considered significant when $p \leq 0.001$.

Tableau 14a : Résultats de l'analyse de variance montrant l'importance des effets simples et combinés de l'addition d'un mélange de additifs (carvacrol et tétrasodium pyrophosphate) et de l'atmosphère d'emballage sur la sensibilité à l'irradiation de *E. coli* et *S. typhi* dans du bœuf haché

Facteurs	DF[1]	P (F > Fcal)[2]	
		E. coli	*S. typhi*
Additifs	1	< 0,001	< 0,001
Atmosphère	3	< 0,001	< 0,001
Additifs * atmosphère	3	< 0,001	< 0,001

[1]Degré de liberté

[2]Effets simples et combinés sont considérés significatif quand $p \leq 0,001$

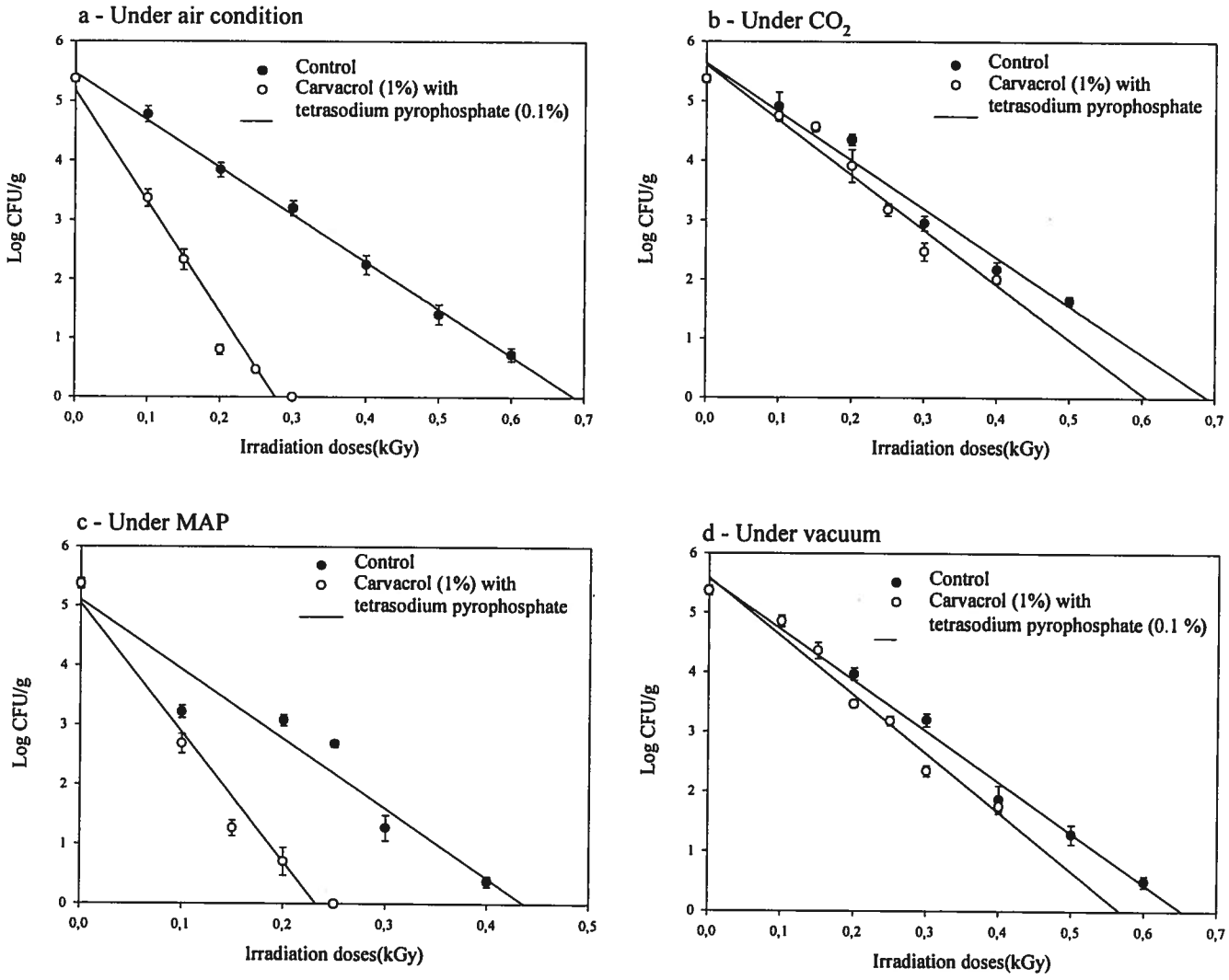


Figure 11 : Irradiation sensitivity of *E. coli* in presence of carvacrol (1%) with tetrasodium pyrophosphate (0.1%) in ground beef under different atmospheres

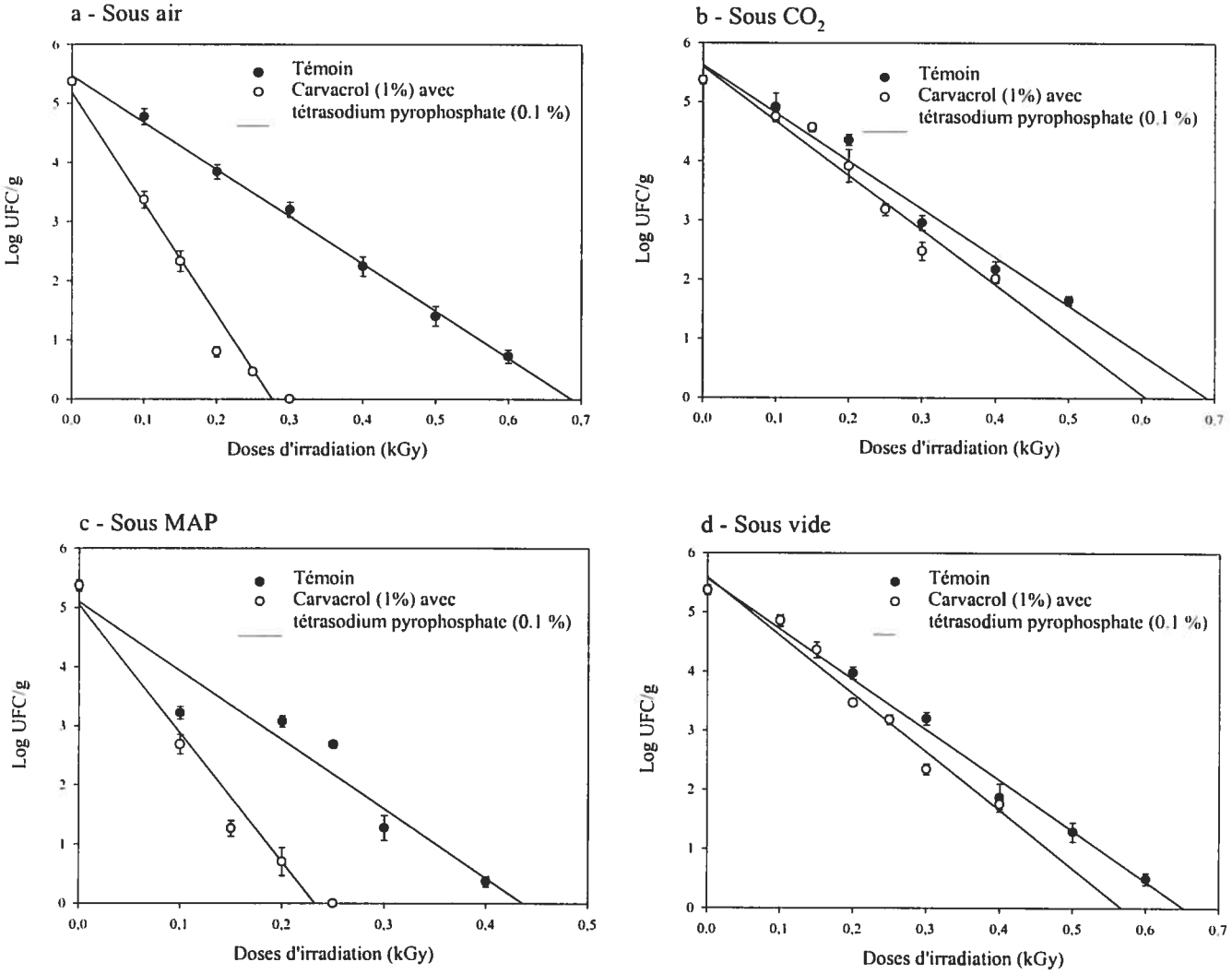


Figure 11a : Sensibilité de *E. coli* à l'irradiation en présence de carvacrol (1%) et tétrasodium pyrophosphate (0.1%) dans du boeuf haché sous differentes atmosphères

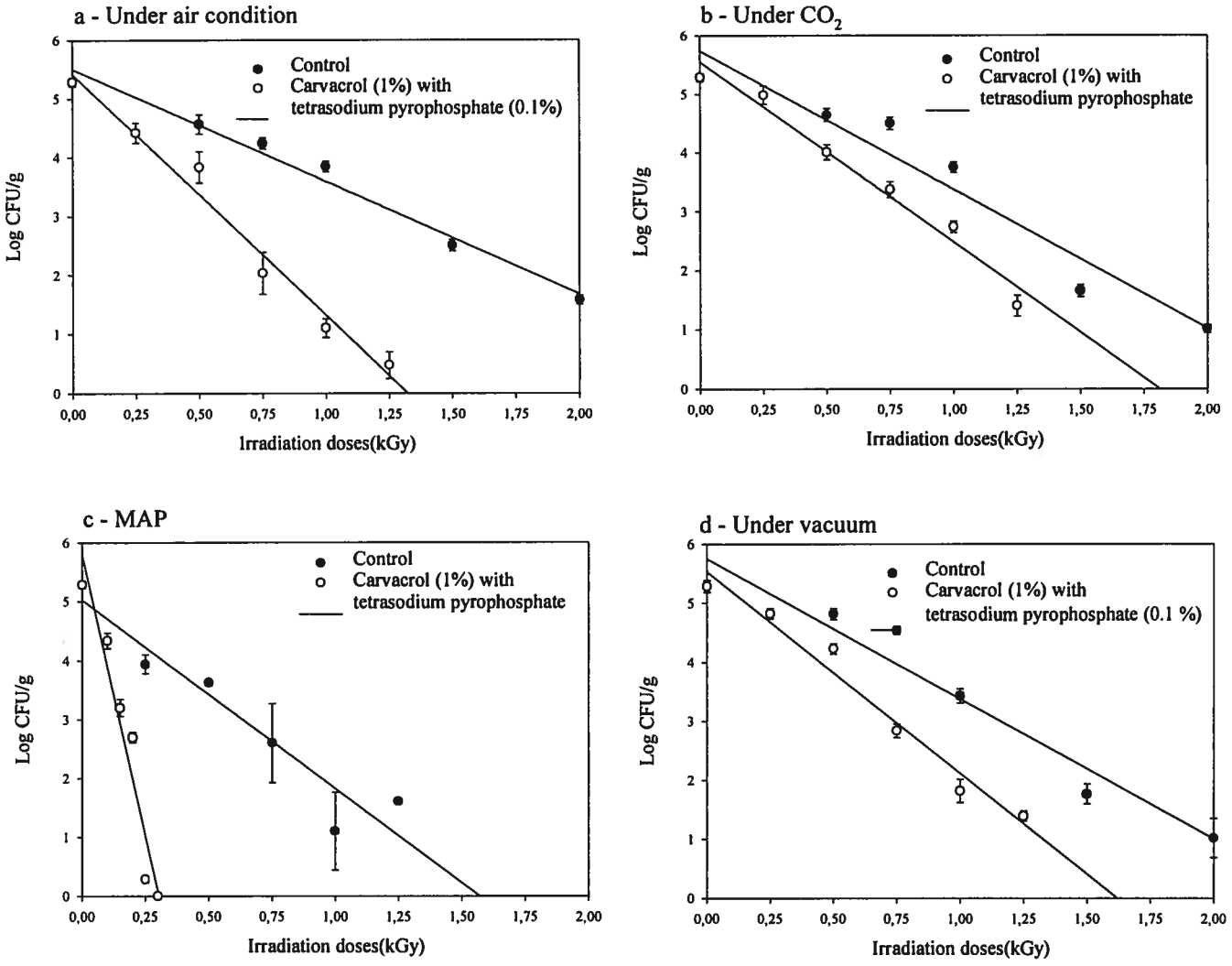


Figure 12 : Irradiation sensitivity of *S. typhi* in presence of carvacrol (1%) with tetrasodium pyrophosphate (0.1%) in ground beef under different atmospheres

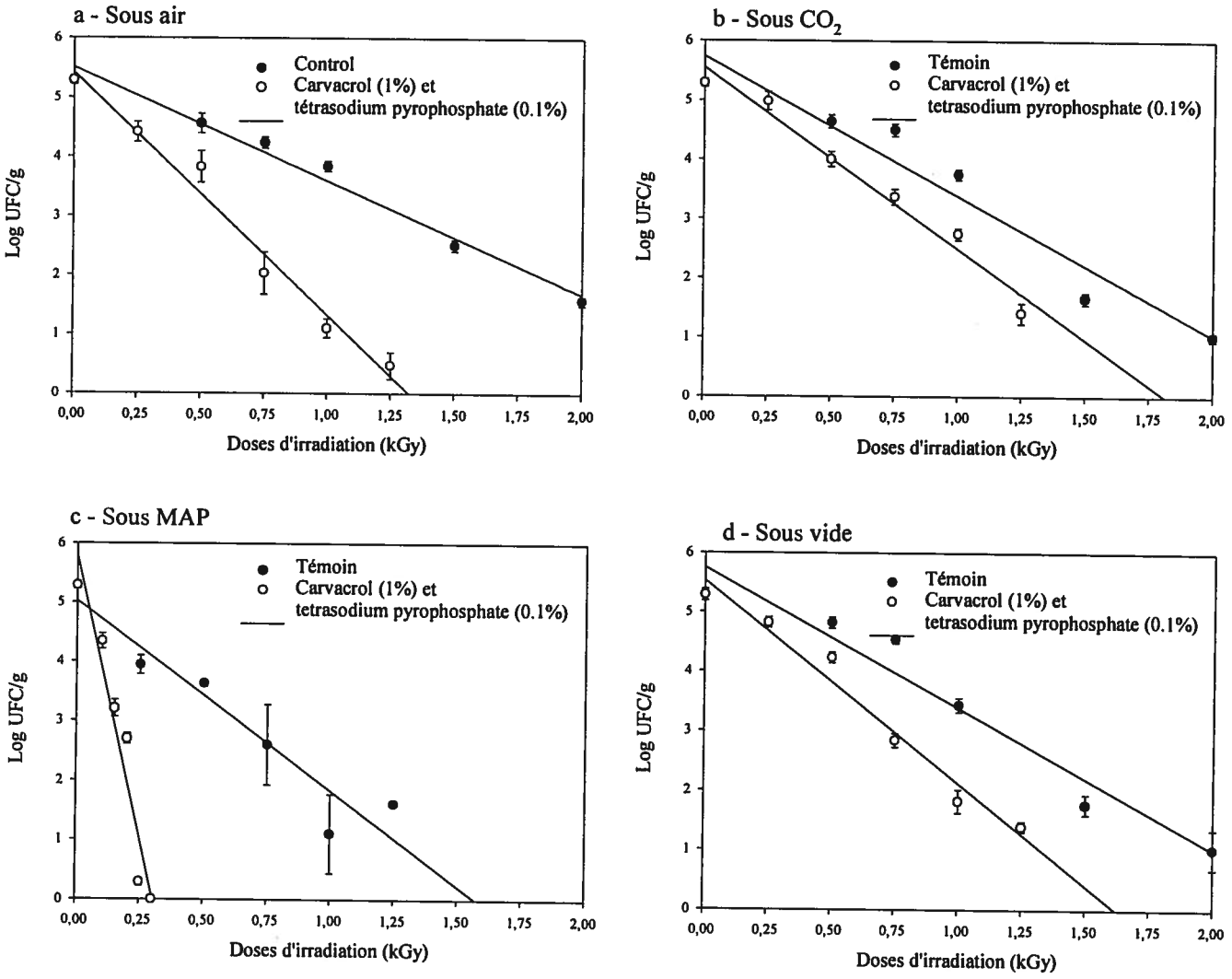


Figure 12a : Sensibilité de *S. typhi* à l'irradiation en présence de carvacrol (1%) et tétrasodium pyrophosphate (0.1%) dans du boeuf haché sous différentes atmosphères

4.6. Determination of the irradiation sensitivity of *E. coli* and *S. typhi* under frozen condition in the presence of the best combination of additives

4.6.1. *Escherichia coli*

Table 15 and figure 13 show the irradiation sensitivity (D_{10}) of *E. coli* in ground beef packed under air containing a mixture of carvacrol and tetrasodium pyrophosphate and stored under refrigerated (4°C) or frozen conditions (- 80°C). According to these results, the D_{10} value of *E. coli* under frozen conditions was 0.227 kGy, which was significantly higher ($p \leq 0.05$) than under refrigerated conditions, where the D_{10} value was 0.126 kGy. When carvacrol and tetrasodium pyrophosphate were added to the ground beef, the irradiation sensitivity was also significantly higher ($p \leq 0.05$) under frozen conditions compared to refrigerated conditions. The D_{10} values were 0.128 kGy and 0.05 kGy respectively. However, results suggest that the addition of the additives to the frozen samples helped to counteract the protective effect of frozen conditions against irradiation treatment.

According to figure 13, irradiation doses of 0.3 and 0.7 kGy were necessary to reduce *E. coli* under the detection level in the presence of additives at 4°C and –80°C respectively. Without additives, *E. coli* was undetectable only at 0.7 kGy when samples were stored at 4°C. At –80°C, a presence of $10^{2.5}$ CFU/g was observed when samples without additives were treated at 0.8 kGy. These result suggest that the addition of additives in ground beef was able to reduce 2.5 times the irradiation dose necessary to reduce *E. coli* under the detection level at 4°C.

4.6.2. *Salmonella typhi*

Table 15 and figure 14 show the irradiation sensitivity (D_{10}) of *S. typhi* in ground beef containing a mixture of carvacrol and tetrasodium pyrophosphate, packed under air and stored under refrigerated or frozen conditions. The addition of additives reduced the irradiation dose necessary to eliminate *S. typhi*. The D_{10} values were reduced from 0.526 to 0.254 kGy at 4°C and from 0.701 kGy to 0.297 kGy at –80°C. These results suggest that the addition of additives reduced the D_{10} by 51.7 % at 4°C and by 57.6 % at –80°C. According to figure 14, *S. typhi* was undetectable in the presence of additives at around 1.3 kGy at 4°C and at 1.5 kGy at –80°C compared to around 2.8 kGy at 4°C without additives. Even 3 kGy was not sufficient to reduce *S. typhi* under the detection level in frozen ground beef.

Table 15 : Irradiation sensitivity of *E. coli* and *S. typhi* in ground beef packed under air as affected by carvacrol (1.0 %) and tetrasodium pyrophosphate (0.1 %) under refrigerated and frozen conditions

Irradiation temperature	Irradiation sensitivity D_{10} (kGy)[1,2]			
	E. coli		*S. typhi*	
	Control	Carvacrol (1 %) + tetrasodium pyrophosphate (0.1 %)	Control	Carvacrol (1 %) + tetrasodium pyrophosphate (0.1 %)
4°C	0.126 ± 0.0036^{a}	$0.055 \pm 0.0014^{a*}$	0.526 ± 0.0161^{a}	$0.254 \pm 0.0102^{a*}$
- 80°C	0.227 ± 0.0092^{b}	$0.128 \pm 0.0052^{b*}$	0.701 ± 0.0100^{b}	$0.297 \pm 0.0164^{b*}$

[1]Duncan – [abcde] Values in same column with different letters are significantly different ($p \leq 0.05$)

[2] For each treatment group (control or Carvacrol + tetrasodium pyrophosphate), means of irradiated samples with asterisks (*) are significantly different ($p \leq 0.05$) from samples without additives.

Tableau 15a : Sensibilité de *E. coli* et *S. typhi* à l'irradiation dans du bœuf haché emballé sous air en présence de carvacrol (1,0 %) et de tétrasodium pyrophosphate (0,1 %) à 4°C et à –80°C

Température d'irradiation	Sensibilité à l'irradiation D_{10} (kGy)[1,2]			
	E. coli		*S. typhi*	
	Témoin	Carvacrol (1 %) + tétrasodium pyrophosphate (0,1 %)	Témoin	Carvacrol (1 %) + tétrasodium pyrophosphate (0,1 %)
4°C	$0,126 \pm 0,0036^{a}$	$0,055 \pm 0,0014^{a*}$	$0,526 \pm 0,0161^{a}$	$0,254 \pm 0,0102^{a*}$
- 80°C	$0,227 \pm 0,0092^{b}$	$0,128 \pm 0,0052^{b*}$	$0,701 \pm 0,0100^{b}$	$0,297 \pm 0,0164^{b*}$

[1] Duncan – [abcde] Valeurs dans la même colonne avec des lettres différentes sont significativement différentes ($p \leq 0,05$)

Pour chaque groupe de traitement (témoin ou carvacrol + tétrasodium pyrophosphate), les valeurs de D_{10} des échantillons avec additifs avec un astérix (*) sont significativement différentes ($p \leq 0,05$) des échantillons sans additifs

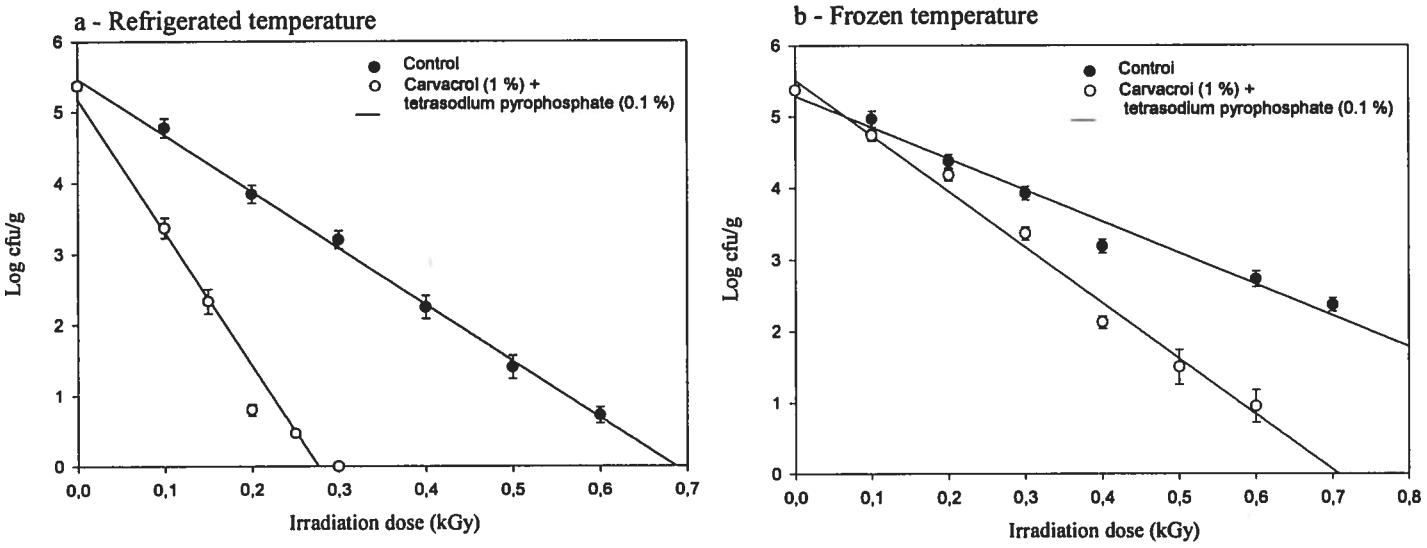


Figure 13 : Irradiation sensitivity of *Escherichia coli* in ground beef packed under air as affected by carvacrol (1.0 %) and tetrasodium pyrophosphate (0.1 %) under refrigerated or frozen conditions (- 80°C)

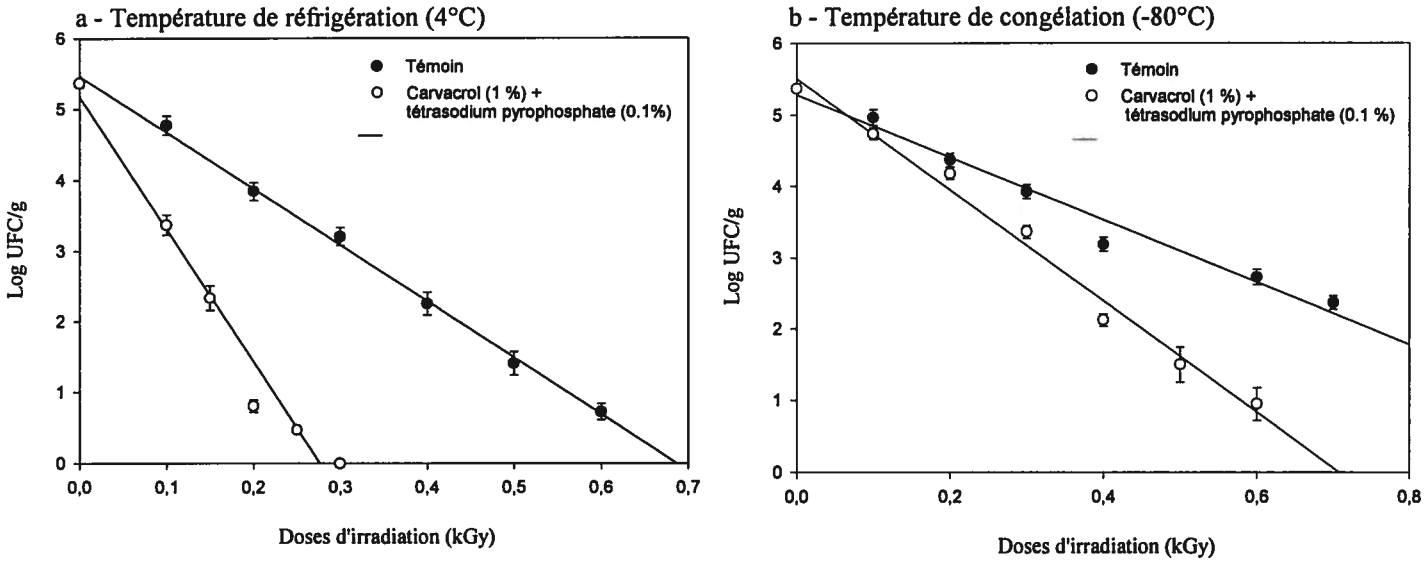


Figure 13a : Sensibilité de *Escherichia coli* à l'irradiation dans du bœuf haché emballé sous air en présence de carvacrol (1,0 %) et de tétrasodium pyrophosphate (0,1 %) à 4°C et à -80°C

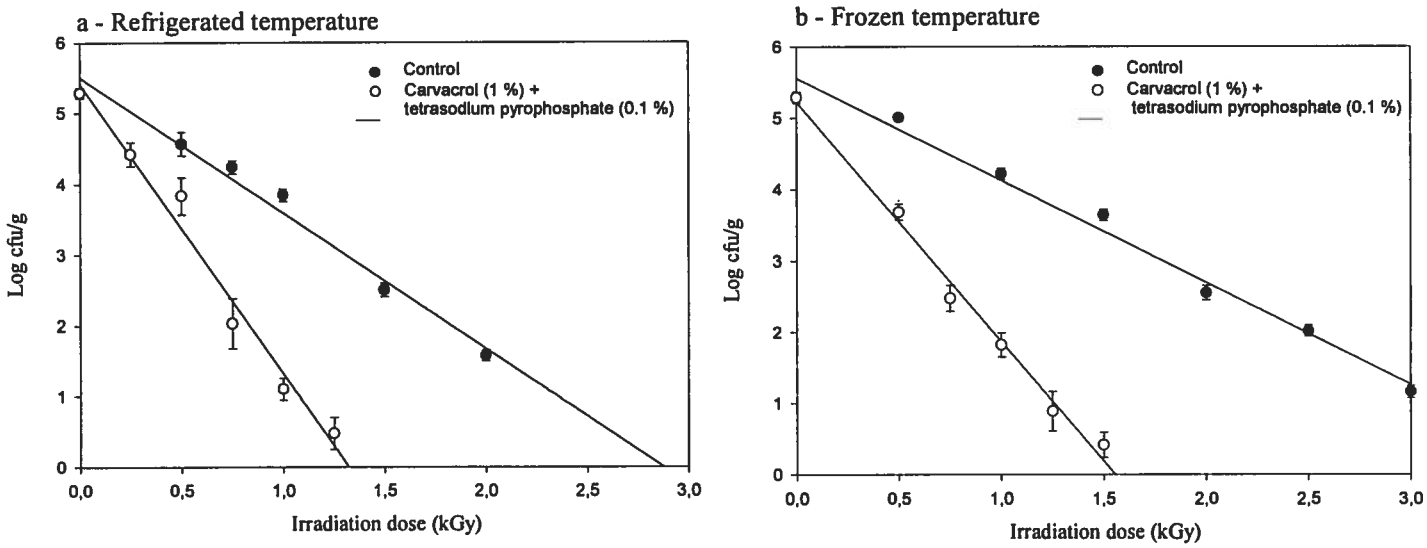
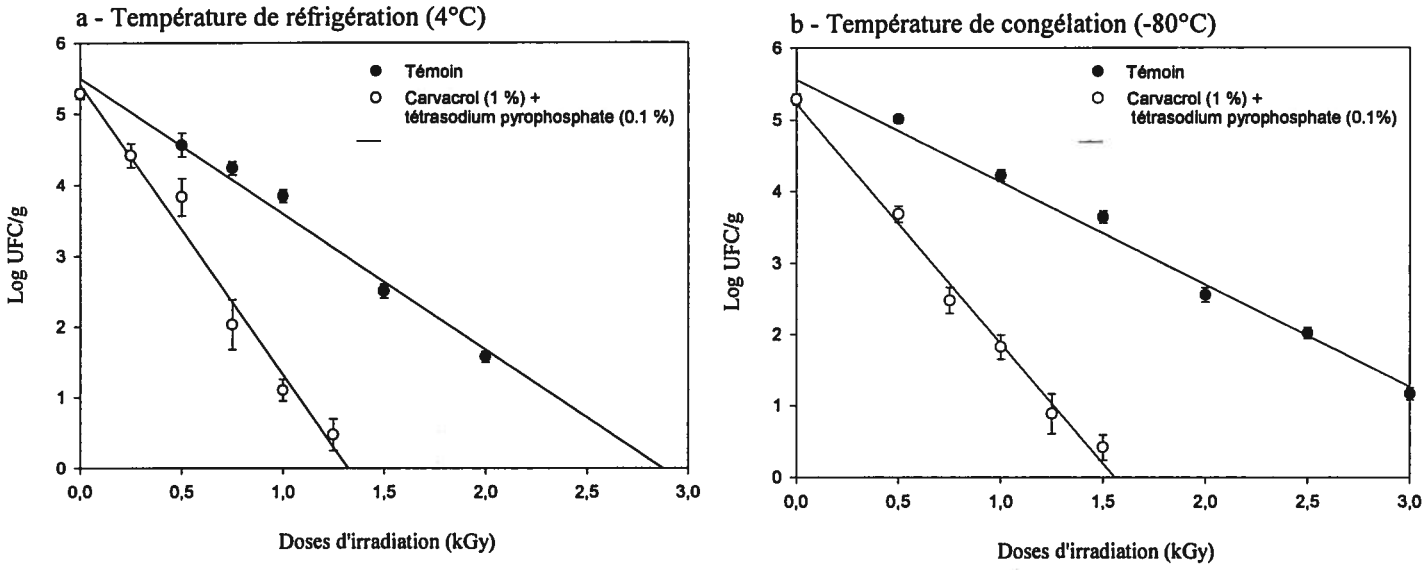


Figure 14 : Irradiation sensitivity of *Salmonella typhi* in ground beef packed under air as affected by carvacrol (1.0 %) and tetrasodium pyrophosphate (0.1 %) under refrigerated or



frozen conditions (- 80°C)

Figure 14a : Sensibilité de *Salmonella typhi* à l'irradiation dans du bœuf haché emballé sous air en présence de carvacrol (1,0 %) et de tétrasodium pyrophosphate (0,1 %) à 4°C et à -80°C

4.7. Determination of lipid oxidation

4.7.1. Effect of various additives on the TBARS content

Table 16 shows the effect of the addition of various additives in unirradiated and irradiated ground beef on the TBARS content. The results showed that when carvacrol, ascorbic acid or tetrasodium pyrophosphate were added to the ground beef, the TBARS value was reduced significantly. In unirradiated samples, best values were obtained for samples in presence of ascorbic acid with TBARS values of 1.102 μM/g compared to 1.915 μM/g for the control. TBARS values of 1.411 and 1.583 μM/g were respectively obtained for carvacrol and tetrasodium pyrophosphate. When carvacrol was mixed with ascorbic acid and tetrasodium pyrophosphate or only with tetrasodium pyrophosphate, the TBARS values was reduced to 1.623 μM/g and 1.641 μM/g respectively, but no significant difference ($p > 0.05$) was observed for both mixtures. Results also showed that when carvacrol was mixed with ascorbic acid, the TBARS value was increased significantly ($p \leq 0.05$), being 2.837 μM/g compared to 1.915 μM/g for the control.

When samples were irradiated, data showed that ascorbic acid, carvacrol and tetrasodium pyrophosphate had inhibited TBARS production. The best values were obtained for samples containing tetrasodium pyrophosphate (1.425 μM/g), ascorbic acid (1.501 μM/g), the mixture of carvacrol and tetrasodium pyrophosphate (1.509 μM/g) and the mixture of carvacrol, ascorbic acid and tetrasodium pyrophosphate (1.641 μM/g) compared to 2.469 μM/g for irradiated samples without additives. A value of 1.770 μM/g was observed for samples containing carvacrol alone. No significant difference ($p > 0.05$) was observed between samples containing carvacrol and samples containing the mixture of all three additives. There was also no significant different ($p > 0.05$) between samples containing the mixture of carvacrol and ascorbic acid (2.542 μM/g) and the control (2.469 μM/g).

4.7.2. Effect of atmospheric conditions on the TBARS content

Table 17 shows the combined effect of the addition of a mixture of carvacrol and tetrasodium pyrophosphate and packaging conditions on the thiobarbituric acid reactive substances (TBARS) content of irradiated ground beef at a dose of 1 kGy.

4.7.2.1.Samples without additives

In unirradiated samples without additives, the lowest TBARS value was obtained for samples packed under vacuum, with TBARS value of 0.977 μM/g compared to 1.915 μM/g for the control samples packed under air. When samples were packed under CO_2 or MAP condition, TBARS values were significantly higher ($p \leq 0.05$), with values of 1.488 μM/g and 2.961 μM/g respectively. These results suggest that the presence of air or MAP affected significantly ($p \leq 0.05$) the TBARS value. Also, samples packed under vacuum, under CO_2 and air at –80°C helped to reduce the TBARS content in ground beef. Values were respectively 0.977 μM/g, 1.488 μM/g and 1.727 μM/g.

The irradiated samples showed that irradiation increased significantly ($p \leq 0.05$) TBARS values of the control from 1.915 μM/g to 2.469 μM/g. Irradiation under MAP or CO_2 had no effect ($p > 0.5$) on TBARS values compared to TBARS compared to unirradiated samples. The values were respectively 3.026 μM/g and 1.458 μM/g, for both treatments. Vacuum condition increased significantly ($p \leq 0.05$) TBARS value from 0.977 μM/g to 1.373 μM/g. Also, samples treated under air, at –80°C and 4°C had a similar values of 2.395 μM/g and 2.469 μM/g, respectively. These results suggest that the irradiation done under vacuum or under CO_2 helped to inhibit the TBARS production, with TBARS values of 1.373 μM/g and 1.458 μM/g respectively.

4.7.2.2.Samples containing additives

When additives were added to the samples, results obtained in unirradiated samples showed that the lowest values were obtained for samples packed under MAP or under vacuum with values of 0.808 μM/g and 0.915 μM/g respectively as compared to 1.641 μM/g for samples packed under air at 4°C. A value of 1.251 μM/g was obtained for samples packed under CO_2 conditions and 1.415 μM/g for samples packed under air at – 80°C. These values were significantly lower than 1.641 μM/g obtained for samples stored under air at 4°C. These results suggest that MAP, CO_2, vacuum and air at –80°C in presence of additives had a significant protective effect ($p \leq 0.05$) against TBARS production. In unirradiated samples, MAP and vacuum were the most effective treatments.

When samples containing additives were irradiated, the best results were obtained for samples packed under MAP (1.138 μM/g) and for samples packed under CO_2 (1.285 μM/g). There was no significant difference ($p > 0.05$) between air at 4°C, air at –80°C, and CO_2. The TBARS values were respectively 1.509μM/g, 1.484 μM/g and 1.285 μM/g. No significant difference ($p > 0.05$) was also observed between vacuum, air at 4°C and air at –80°C, with TBARS values of 1.681 μM/g, 1.509 μM/g and 1.484 μM/g. These results showed that the most effective packaging conditions in presence of additives were MAP and CO_2.

Table 18 show the results of the variance analysis on the significance of simple and combined effects of the addition of the mixture of carvacrol and tetrasodium pyrophosphate; the packaging atmosphere and irradiation on the TBARS content of ground beef. According to the results, the additives, the packaging atmosphere or the irradiation treatment had a significant effect ($p \leq 0.001$) on the TBARS content. An interaction was observed between the addition of the additives and the packaging atmosphere ($p \leq 0.001$); between the additives and the irradiation treatment ($p \leq 0.05$); and between the packaging atmosphere and the irradiation treatment ($p \leq 0.001$).

Table 16 : Effect of the addition of various additives on TBARS content in unirradiated and irradiated ground beef packed under air

Additives	TBARS (μM/g)[1,2]	
	Unirradiated	Irradiated (1 kGy)
Control	1.915 ± 0.193^{d}	$2.469 \pm 0.172^{c*}$
Ascorbic acid (0.5 %)	1.102 ± 0.107^{a}	$1.501 \pm 0.104^{a*}$
Carvacrol (1.0 %)	1.411 ± 0.221^{b}	$1.770 \pm 0.189^{b*}$
Tetrasodium pyrophosphate (0.1 %)	1.583 ± 0.246^{bc}	1.425 ± 0.070^{a}
Carvacrol (1.0 %) + ascorbic acid (0.5 %) + tetrasodium pyrophosphate (0.1 %)	1.623 ± 0.206^{c}	1.641 ± 0.257^{ab}
Carvacrol (1.0 %) + tetrasodium pyrophosphate (0.1 %)	1.641 ± 0.218^{c}	1.509 ± 0.262^{a}
Carvacrol (1.0 %) + ascorbic acid (0.5 %)	2.837 ± 0.202^{e}	2.542 ± 0.304^{c}

[1]Duncan – [abcde] Values in same column with different letters are significantly different ($p \leq 0.05$)
[2] For each treatment group (control or Carvacrol + tetrasodium pyrophosphate), means of irradiated samples with asterisks (*) are significantly different ($p \leq 0.05$) from corresponding unirradiated samples.

Tableau 16a : Effet de l'addition de différents additifs dans du bœuf haché non-irradié et irradié emballé sous air sur l'oxydation des gras

Additifs	TBARS (µM/g)[1,2]	
	Non-irradié	Irradié (1 kGy)
Témoin	$1,915 \pm 0,193^{d}$	$2,469 \pm 0,172^{c*}$
Acide ascorbique (0,5 %)	$1,102 \pm 0,107^{a}$	$1,501 \pm 0,104^{a*}$
Carvacrol (1,0 %)	$1,411 \pm 0,221^{b}$	$1,770 \pm 0,189^{b*}$
Tétrasodium pyrophosphate (0,1 %)	$1,583 \pm 0,246^{bc}$	$1,425 \pm 0,070^{a}$
Carvacrol (1,0 %) + acide ascorbique (0,5 %) + tétrasodium pyrophosphate (0,1 %)	$1,623 \pm 0,206^{c}$	$1,641 \pm 0,257^{ab}$
Carvacrol (1,0 %) + tétrasodium pyrophosphate (0,1 %)	$1,641 \pm 0,218^{c}$	$1,509 \pm 0,262^{a}$
Carvacrol (1,0 %) + acide ascorbique (0,5 %)	$2,837 \pm 0,202^{e}$	$2,542 \pm 0,304^{c}$

[1] Duncan – [abcde] Valeurs dans la même colonne avec une lettre différentes sont significativement différent ($p \leq 0,05$)

[2] Pour chaque groupe de traitement (témoin ou carvacrol + tétrasodium pyrophosphate), les moyennes des échantillons irradiés avec un astérix (*) sont significativement différent ($p \leq 0,05$) des échantillons correspondant non-irradiés

Table 17: Effect of the addition of various additives on TBARS content in unirradiated and irradiated (1 kGy) ground beef packed under various atmosphere (CO_2 and MAP) and under vacuum

Atmosphere	TBARS (µM/g)[1,2]			
	Control		Carvacrol (1 %) + tetrasodium pyrophosphate (0.1%)	
	Unirradiated	Irradiated	Unirradiated	Irradiated
Vacuum	0.977 ± 0.107^{a}	$1.373 \pm 0.209^{a*}$	0.915 ± 0.141^{a}	$1.681 \pm 0.306^{c*}$
CO_2	1.488 ± 0.099^{b}	1.458 ± 0.096^{a}	1.251 ± 0.221^{b}	1.285 ± 0.215^{ab}
Air -80°C	1.727 ± 0.210^{c}	$2.395 \pm 0.175^{b*}$	1.415 ± 0.172^{b}	1.484 ± 0.264^{bc}
Air 4°C (control)	1.915 ± 0.193^{d}	$2.469 \pm 0.172^{b*}$	1.641 ± 0.218^{c}	1.509 ± 0.262^{bc}
MAP[3]	2.961 ± 0.188^{e}	3.026 ± 0.126^{c}	0.808 ± 0.053^{a}	$1.138 \pm 0.246^{a*}$

[1] [abcde] Values in same column with different letters are significantly different ($p \leq 0.05$)
[2] For each treatment group (control or Carvacrol + tetrasodium pyrophosphate), means of irradiated samples with asterisks (*) are significantly different ($p \leq 0.05$) from corresponding unirradiated samples.
[3] MAP : 60 % O_2 – 30 % CO_2 – 10 % N_2

Tableau 17a: L'effet de l'ajout d'un mélange de carvacrol (1%) avec du tétrasodium pyrophosphate (0.1 %) dans du bœuf haché non-irradié et irradié (1 kGy) emballé sous différents atmosphères (CO_2 et MAP) et sous vide sur l'oxydation des gras

Atmosphère	TBARS (µM/g)[1,2]			
	témoin		Carvacrol (1 %) + tétrasodium pyrophosphate (0,1%)	
	Non-irradié	Irradié	Non-irradié	Irradié
Sous vide	$0,977 \pm 0,107^{a}$	$1,373 \pm 0,209^{a*}$	$0,915 \pm 0,141^{a}$	$1,681 \pm 0,306^{c*}$
CO_2	$1,488 \pm 0,099^{b}$	$1,458 \pm 0,096^{a}$	$1,251 \pm 0,221^{b}$	$1,285 \pm 0,215^{ab}$
Air -80°C	$1,727 \pm 0,210^{c}$	$2,395 \pm 0,175^{b*}$	$1,415 \pm 0,172^{b}$	$1,484 \pm 0,264^{bc}$
Air 4°C (témoin)	$1,915 \pm 0,193^{d}$	$2,469 \pm 0,172^{b*}$	$1,641 \pm 0,218^{c}$	$1,509 \pm 0,262^{bc}$
MAP[3]	$2,961 \pm 0,188^{e}$	$3,026 \pm 0,126^{c}$	$0,808 \pm 0,053^{a}$	$1,138 \pm 0,246^{a*}$

[1] Duncan - [abcde] Valeurs dans la même colonne avec une lettre différentes sont significativement différentes ($p \leq 0,05$)
[2] Pour chaque groupe de traitement (témoin ou carvacrol + tétrasodium pyrophosphate), les moyennes des échantillons irradiés avec un astérix (*) sont significativement différent ($p \leq 0,05$) des échantillons correspondant non-irradiés
[3] MAP : 60 % O_2 – 30 % CO_2 – 10 % N_2

Table 18 : Results of variance analysis showing the significance of simple and combined effects of the addition of the mixture of additives (carvacrol with tetrasodium pyrophosphate), the packaging atmosphere and irradiation on the TBARS content of ground beef

Factors	DF[1]	P (F > Fcal)[2]
		TBARS
Additives	1	< 0.001**
Atmosphere	4	< 0.001**
Irradiation	1	< 0.001**
Additives x atmosphere	4	< 0.001**
Additives x irradiation	1	0.012*
Atmosphere x Irradiation	4	< 0.001**
Additives x atmosphere x irradiation	4	0.643

[1] Degree of freedom

[2] * Simple and combined effects are considered significant when $p \leq 0.05$.

** Simple and combined effects are considered significant when $p \leq 0.001$.

Tableau 18a : Résultats de l'analyse de variance montrant l'importance des effets simples et combinés de l'addition d'un mélange d'additifs (carvacrol et tétrasodium pyrophosphate), de l'atmosphère d'emballage et de l'irradiation sur le contenu en TBARS du bœuf haché

Facteurs	DF[1]	P (F > Fcal)[2]
		TBARS
Additifs	1	< 0,001**
Atmosphère	4	< 0,001**
Irradiation	1	< 0,001**
Additifs x Atmosphère	4	< 0,001**
Additifs x irradiation	1	0,012*
Atmosphère x Irradiation	4	< 0,001**
Additifs x Atmosphère x irradiation	4	0,643

[1] Degré de liberté

[2] * Effets simples et combinés sont considérés significatif lorsque $p \leq 0,05$,

** Effets simples et combinés sont considérés significatif lorsque $p \leq 0,001$,

5. Discussion

5.1. MICs for individual additives in ground beef

The addition of various additives has been proven to help control the bacterial population in many food products. But not all of them can be used in ground beef. Some may require a high concentration to obtain the desire effect and some may give a strong odour to the meat.

All of the additives tested in this study had either antimicrobial and/or antioxidant properties. All the additives tested in this experiment for the MIC have been reported by different authors to have inhibitory effect on some bacteria tested in broth or in different food product. For *E. coli*, the three most effective additives in reducing the bacterial population by 1 log were carvacrol, thymol and trans-cinnamaldehyde, with MIC values of 0.88 %, 1.14 % and 1.57 % respectively. For *S. typhi*, these additives were also the most efficient and the concentrations needed to reduce by 1 log were 0.89 % for trans-cinnamaldehyde, 1.15 % for carvacrol and 1.6 % for thymol. The greatest effectiveness of carvacrol, thymol and trans-cinnamaldehyde was to be expected since these additives have been reported to be the active molecules responsible of the antibacterial activity in many essential oils (Kim *et al.*, 1995b; Suresh *et al.*, 1992; Ouattara *et al.*, 1997; Lacroix, *et al*, 1997; Mahrour *et al.*, 1998).

The effectiveness of carvacrol, thymol and trans-cinnamaldehyde against *E. coli* and *S. typhi* in ground beef can be explained by the mechanism of inhibition, evaluated by Helander *et al* (1998), on *E. coli* O157:H7 and *S. typhimurium*. Carvacrol was shown to be effective in inhibiting bacterial growth at very low concentration (1 – 3 mM). The effect of carvacrol on the cell surface and on the concentration of intracellular and extracellular ATP has been shown to decrease the intracellular ATP (Helander *et al.*, 1998; Ultee *et al.*,1999). This would result in a decrease in energy for the cell (Pelmont, 1993). This would in turn slow down the cellular metabolism. A decrease in intracellular ATP could be related to an increase in extracellular ATP (Hellander *et al.*, 1998), but this has not been proven. However, the depletion of intracellular ATP was associated with a change in the membrane potential (Hellander *et al.*, 1998).

Carvacrol was also responsible for the disruption of the permeability of the cytoplasmic membrane (Helander *et al.*, 1998; Ultee *et al.*,1999). An interaction between carvacrol and the membrane was created, changing the permeability for cations like H^+ and K^+. The change in the cell gradient lead to the impairment of the essential process in the bacterial cell, leading to the cell death. The effect of thymol and trans-cinnamaldehyde was also studied by Helander *et al* (1998). In their experiment, they showed that thymol and trans-cinnamaldehyde were just as efficient in reducing *E. coli* and *S. typhimurium* as carvacrol. Thymol, just like carvacrol helped to decrease the intracellular ATP and an increase in extracellular ATP. This would indicate that the cytoplasmic membrane of bacteria was affected.

When trans-cinnamaldehyde was added, the amount of intracellular and extracellular ATP was not affected, which means that this additive did not affect the permeability of the membranes (Hellander *et al.*, 1998). The addition of trans-cinnamaldehyde had no disintegrative effects on the outer membranes of the cell. This could indicate that to inhibit bacteria in the presence of trans-cinnamaldehyde, it has to gain access to the periplasm or other deeper parts of the cell. The inability for trans-cinnamaldehyde to affect the outer membranes like carvacrol or thymol, can be explain by the absence of the phenolic character. Phenols are known for their abilities to disrupt normal activity of the membranes.

As for the essential oils tested, thyme was the most effective against both bacteria, with a concentration of 2.33 % for *E. coli* and 2.75 % for *S. typhi* to reduce by 1 log the bacterial population. In the presence of rosemary, the concentration necessary to reduce the bacterial population by 1 log in ground beef increased to 10.37 % for *E. coli* and to 13.56 % for *S. typhi*. The difference in concentration can be explained by the presence of thymol and carvacrol, in thyme. These additives have been known to have a very strong antimicrobial effect. Rosemary is used mainly for its antioxidant properties. It was to be expected that thyme and rosemary were less effective than carvacrol thymol and trans-cinnamaldehyde, since the essential oils are a mixture of various fractions. The fractions with the antimicrobial activity are therefore diluted.

Ascorbic acid produced 1 log CFU/g reduction of the bacterial population at the concentrations of 1.83 % for *S. typhi* and 2.71 % for *E. coli*. However, previous experiments done in our laboratory demonstrated that the presence of more than 0.5 % of ascorbic acid in ground beef had detrimental effect on the sensorial characteristics (Giroux *et al.*, 2001). However, the addition of 0.5 % of ascorbic acid helps to preserve the colour and the odour of the meat following the irradiation treatment.

Tannic acid had no inhibitory effect on *E. coli* and *S. typhi* when added to ground beef. A concentration of 11.15 % for *E. coli* and 21.18 % for *S. typhi* was necessary to reduce the bacterial population by 1 log in ground beef. These high concentrations were not surprising, considering that tannic acid was found to only have an inhibitory effect in broth, against several intestinal bacteria (Chung *et al.*, 1998). The addition of tannic acid will slow bacterial growth due to its strong iron binding capacity. However, bacterial growth could be restore by adding iron to the growing media. The presence of iron in ground beef was probably responsible of the low efficiency of tannic acid against *E. coli* and *S. typhi.*

Of all the additives tested, the commercial products, Duralox and Herbalox, had the least effect on *E. coli* and *S. typhi*. The absence of inhibitory effect of these commercial products was to be expected since these products were extracted from the same plant as the rosemary extract used. The antimicrobial fractions of the commercial products are too diluted to have an effect on either *E. coli* or *S. typhi*.

5.2. Irradiation sensitivity of *E. coli* and *S. typhi* in the presence of various additives

Ground beef has been associated with several cases of food poisoning due to the presence of foodborne pathogens. Irradiation is being considered as the solution to help reduce or eliminate the bacteria responsible for the spoilage of ground beef. The addition of some additives will help to reduce the irradiation dose necessary to control the bacterial population of the meat.

Among the additives tested on the irradiation sensitivity of *E. coli* and *S. typhi*, the strongest synergistic effect was observed with the additives with antimicrobial properties. For both bacteria, the most effective additives were trans-cinnamaldehyde, thymol, carvacrol and thyme, at concentrations corresponding to the MIC in ground beef. The effectiveness of trans-cinnamaldehyde, thymol and carvacrol during irradiation can be explained by the fact that these three additives were effective in reducing *E. coli* and *S. typhi* before irradiation in ground beef (section 4.1). The addition of carvacrol and thymol to the ground beef decreased the intracellular ATP, meaning a reduction in energy, which is vital for the cell to function properly (Helander *et al.*, 1998; Ultee *et al.*,1999). So the addition of these additives may have made the bacteria more sensitive to the lethal effects of irradiation. With less energy available, the bacteria cell was unable to make the necessary repairs needed to survive after the irradiation treatment. When the concentration of the additives was reduced, the synergistic effect was also smaller, since the inhibitory effect of these additives was proportional with the concentration added.

Thyme was among the best additive in increasing the irradiation sensitivity of both bacteria. This can be explained by the presence of thymol and carvacrol as components of thyme. Again, the irradiation sensitivity of both bacteria increased with an increase in concentration of thyme in ground beef.

The addition of tannic acid helped to increase the irradiation sensitivity of both bacteria at a concentration of 0.38 %. This increase in sensitivity was surprising, considering that this additive showed no inhibitory effect in ground beef without irradiation (MIC in ground beef evaluated at 11.15 % for *E. coli* and at 21.18 % for *S. typhi*). The addition of tetrasodium pyrophosphate also increased the irradiation sensitivity, but only for *S. typhi* and had no effect on *E. coli*. Normally, this particular phosphate has no antimicrobial properties, it only helps with the water holding, increasing the tenderness of the meat (Ellinger, 1972).

When nisin was added to the ground beef, the irradiation sensitivity of both bacteria was increased. Those results were unexpected. It was reported in literature that nisin did not inhibit Gram negative bacteria, that it was only effective against Gram positive bacteria (Delves-Broughton, (1990). This means that the gamma rays weaken the bacterial cell just enough to let the nisin penetrate the outer membrane. Normally, a chelating agent, EDTA, needs to be added with nisin to improve the sensitivity of against Gram negative bacteria (Stevens *et al.*, 1991). The addition of EDTA helps to increase the cellular permeability, resulting in an increase susceptibility to antibiotics.

When EDTA was added alone, this additive helped to increase the irradiation sensitivity of *S. typhi*, but had no effect on the irradiation sensitivity of *E. coli*. When nisin and EDTA were added together to the ground beef, the irradiation sensitivity of both bacteria was increased compared with the control. The combination of nisin and EDTA had, however, a greater effect on *S. typhi*, than on *E. coli*. The efficiency was 35.4 % for *S. typhi* compared to 4 % for *E. coli*. The addition of EDTA to ground beef already containing nisin did not affect the sensitivity of *E. coli*. With *S. typhi*, the addition of EDTA to ground beef containing nisin did help to increase the irradiation sensitivity compared to ground beef with nisin alone. This means that the presence of EDTA increased the cellular permeability of *S. typhi*. With a weaker permeability, nisin was able to penetrate the outer membrane, thus reducing the bacterial count.

Among the additives tested, ascorbic acid, BHA, BHT, carnosine and rosemary, several were antioxidants, which can retard deterioration caused by lipid oxidation during irradiation. These antioxidants react with free radicals generated by radiation, free radicals that would normally react with the bacterial cell. The reduction of free radicals will help to increase the resistance to radiation or will have no effect. Such was the case with ascorbic acid. The addition of carnosine did not effect the irradiation sensitivity of *E. coli*, but it did increase slightly the sensitivity of *S. typhi*. It was in fact reported that the addition of carnosine helped to protect *Aeromonas hydrophila* against radiation (Stechinni *et al.*, 1998). The addition of BHA, BHT and rosemary did increase the sensitivity of both bacteria. Even if it has been demonstrated that these three additives did have some antimicrobial properties, the increase in irradiation sensitivity was, however, lower than the additives with strong antimicrobial properties.

5.3. Selection of additives

Following these results, three additives were selected to test different combination of additives on the irradiation sensitivity of *E. coli* and *S. typhi*. Those additives were carvacrol, ascorbic acid and tetrasodium pyrophosphate. Carvacrol was selected for its antimicrobial properties and the addition of carvacrol did help to increase significantly the irradiation sensitivity of both bacteria. Other additives could have been selected instead, like trans-cinnamaldehyde or thymol. However, with trans-cinnamaldehyde, an inappropriate odour appeared. Also, the concentration needed was smaller with carvacrol. Ascorbic acid was selected for its antioxidant properties which will help to maintain the taste of the meat and the colour following the irradiation treatment. Tetrasodium pyrophosphate was chosen for its water retention abilities, which increases the tenderness of the meat.

5.4. Irradiation sensitivity of *E. coli* and *S. typhi* in the presence of different concentration of carvacrol

This experiment was accomplished to determine if the addition of a smaller concentration would have the same effect on the bacteria as the concentration used earlier. With both bacteria, the irradiation sensitivity of the bacteria increased with the concentration of carvacrol. An increase in irradiation sensitivity was observed even with a very small concentration (0.2 % for *E. coli* and 0.5 % for *S. typhi*). This suggests that the loss of energy may be significant enough to slightly alter the repair mechanism of the cell. When the concentration of carvacrol reached 1.2 % for *E. coli* and 1.75 % for *S. typhi*, the bacteria were completely eliminated from the ground beef after irradiation. The integrity of the cytoplasmic membrane may have been altered and the bacteria could not survive the irradiation treatment, even at very low doses. A higher concentration was necessary to completely eliminate *S. typhi* from the ground beef after irradiation than with *E. coli*. These concentrations were 1.75 % for *S. typhi* and 1.2 % for *E. coli*. This means that *S. typhi* was more resistant to the bactericidal action of carvacrol.

5.5. Best combination of additives for their efficiency on *E. coli* and *S. typhi* reduction during irradiation

The irradiation sensitivity of *E. coli* and *S. typhi* was determined in the presence of carvacrol, a mixture of carvacrol and tetrasodium pyrophosphate, a mixture of carvacrol and ascorbic acid and a mixture of all three additives. The irradiation sensitivity of both bacteria was greater when carvacrol or the mixture of carvacrol and tetrasodium pyrophosphate was added. Since tetrasodium pyrophosphate has no antimicrobial effect, it is therefore normal that no significant difference ($p > 0.05$) was observed in the irradiation sensitivity between the two samples. The increase in sensitivity was due to the presence of carvacrol. With the concentration chosen, both bacteria were affected by the bactericidal action of carvacrol before irradiation. The carvacrol may have decreased the energy level (decrease in ATP inside the cell; Helander *et al.*, 1998; Ultee *et al.*,1999) of the bacterial cell, making it impossible for the cell to make the necessary repairs of the damages caused by the increase of radiation. The addition of carvacrol may have helped to increase the lethal effect of irradiation.

On the other hand, the bactericidal action of carvacrol with irradiation was eliminated or reduced with the addition of another additive, like ascorbic acid. Ascorbic acid, being an antioxidant, protects the bacteria by reacting with the free radicals generated by the radiation treatment that would otherwise react with the bacteria. When the mixture of carvacrol and ascorbic acid was added to the ground beef containing *E. coli*, the irradiation sensitivity was similar to samples without additives and when the mixture of carvacrol, ascorbic acid and tetrasodium pyrophosphate was added, the bacteria were more resistant. This means that the addition of ascorbic acid completely neutralised the radiosensitization effect of carvacrol. For the samples containing *S. typhi*, the addition of the mixture of carvacrol and ascorbic acid or the mixture of carvacrol, ascorbic acid and tetrasodium pyrophosphate increased the irradiation sensitivity of the bacteria, but to a lesser degree than the addition of carvacrol or of the mixture of carvacrol and tetrasodium pyrophosphate. This means that the addition of ascorbic acid counteracted some of the bactericidal action of carvacrol. This could imply a different mechanism of action of carvacrol and ascorbic acid in the two test bacteria.

Following the experiments, it was decided to chose the mixture of carvacrol and tetrasodium pyrophosphate for further study using combination of additives, atmosphere condition and irradiation temperature on the irradiation sensitivity of *E. coli* and *S. typhi*. This mixture was selected for the bactericidal action of carvacrol and for the water retention ability of tetrasodium pyrophosphate that will increase tenderness of the meat.

5.6. Irradiation sensitivity of *E. coli* and *S. typhi* under various atmosphere in the presence of the best combination of additives

It is well established that bacteria are more sensitive to irradiation in the presence of oxygen (Thayer and Boyd, 1999). This is due to the formation of oxygen radicals with a high lethal effect. Regardless of the presence or absence of the mixture of carvacrol and tetrasodium pyrophosphate, *E. coli* and *S. typhi* were more sensible to irradiation under MAP, which contained 60 % of oxygen compared to 20.9 % in air.

When ground beef was packed under vacuum and CO_2 packaging, both bacteria were only slightly more sensitive than under air packaging. Experiments done by Patterson (1988) on chicken showed that under vacuum and CO_2 packaging, the lethal effect caused by radiation was greater than under air packaging. With vacuum packaging, Hastings *et al.* (1986) obtained the same results as our experiment, where the D_{10} values of *Lactobacillus sake* was reduced by less than 1 %, compared to treatment under air. With the lack of oxygen, the production of hydrogen peroxide is very small and increases the irradiation resistance of bacteria (Thakur and Singh, 1994). As for CO_2 packaging, the radicals formed may have lower reducing power than the oxygen radicals (von Sonntag, 1987).

When the mixture of carvacrol and tetrasodium pyrophosphate were added to the ground beef packaged under vacuum and CO_2, *E. coli* and *S. typhi* were both more resistant to irradiation when compared with samples with the mixture of additives under air packaging. This could be due to the fact that under vacuum and CO_2, the slower growth rate and the longer lag phase could mean a reduction in the bactericidal effect of carvacrol. (Hintlian and Hotchkiss, 1986)

5.7. Irradiation sensitivity of *E. coli* and *S. typhi* packed under air and treated under frozen condition in the presence of the best combination of additives

The irradiation sensitivity of *E. coli* and *S. typhi* was greatly reduced under frozen condition compared with refrigerated condition. This was true also for samples with and without the mixture of carvacrol and tetrasodium pyrophosphate. Under frozen condition, the intermediate radicals formed from water radiolysis are known to get trapped in the frozen components of the meat (Thakur and Singh, 1994; OMS, 1995). The mobility of these radical may therefor have been reduced, making it difficult to react with other radicals or the bacteria in the ground beef.

When the mixture of carvacrol and tetrasodium pyrophosphate was added to the ground beef, the lowering of the temperature did not altered the effect of the additives on the bacteria. The addition of the mixture of the additives did, however, increase the irradiation sensitivity compared to the frozen samples without the presence of additives. These results suggest that the mixture may have had a bactericidal action on the bacteria before the meat was completely frozen.

5.8. Effect of lipid oxidation on ground beef

Lipid oxidation is responsible for the development of warnoff flavour in irradiated ground beef. Irradiation will generate the production of free radicals, that react with the double bonds of unsaturated fatty acids to produce hydroperoxides (Decker and Faraji, 1990).

When ground beef samples in the absence of any additive were irradiated, the TBARS content was higher than in unirradiated samples. The increase in TBARS was associated with the formation of hydroperoxide generated by the free radicals formed from the presence of oxygen (Decker and Fariji, 1990; Ouattara, *et al.*, 2002). When the additives were added to ground beef samples, the production of TBARS was reduce in both irradiated samples and unirradiated samples, and no difference ($p > 0.05$) was observed between irradiated and unirradiated samples. This was due to the antioxidant properties of both carvacrol and ascorbic acid. These additives will react with the free oxygen radicals generated during irradiation, reducing the lipid oxidation. Lacroix *et al.* (1997) have demonstrated that the presence of natural antioxidants, like thyme and rosemary, helps to reduce lipid oxidation.

However, the TBARS content was increased in unirradiated samples containing the mixture of carvacrol and ascorbic acid, compared with unirradiated samples without the addition of additives. In irradiated samples, no difference was observed between samples with the mixture of carvacrol and ascorbic acid and samples without the addition of additives. These results were surprising, since both carvacrol and ascorbic acid possess antioxidant properties. The presence of these additives should have reduce the TBARS content in ground beef samples. Mahrour *et al.* (1998) have reported carvacrol as an effective antioxidant in the control of lipid oxidation and Ouattara *et al.* (2002) have demonstrated that the addition of ascorbic acid helps to stabilise lipid oxidation following irradiation treatment.

When ground beef samples were packaged under various atmospheres, the highest TBARS content was with irradiated and unirradiated samples without the presence of additives and packaged under MAP condition. This is due to the high amount of oxygen in the gas mixture (60 % oxygen). Samples packed under CO_2 had lower TBARS content due to lower free radicals production in the presence of CO_2 than in the presence of oxygen (von Sonntag, 1987). When samples were packed under vacuum, the absence of oxygen helped to reduce the TBARS content in unirradiated ground beef samples.

When these samples were irradiated, the TBARS content was also one of the lowest of the irradiated samples. With the lack of oxygen, the production of free radicals does get reduced (Thakur and Singh, 1994). This may means that the lipid oxidation caused by irradiation will be reduced as well. Under air atmosphere and at –80°C, the content of TBARS was reduced compared to samples at 4°C. However, the freezing conditions did not help to protect against lipid oxidation.

When the mixture of carvacrol and tetrasodium pyrophosphate was added to ground beef, the TBARS content was reduce compared with samples without additives. Again, this is due to the antioxidant properties of carvacrol. When the ground beef was packaged under MAP, the TBARS content in irradiated samples was higher than unirradiated samples. This can be again explained by the high concentration of oxygen in the gas mixture.

6. Conclusion

The addition of some additives to ground beef prior to irradiation treatment help to increase the irradiation sensitivity of *E. coli* and *S. typhi*. Among the additives tested, trans-cinnamaldehyde was the most effective in increasing the irradiation sensitivity, followed by thymol, carvacrol and thyme. The efficiency of these additives ranged from 70.6 % to 18.2 % for *E. coli* and from 73.6 % to 50.6 % for *S. typhi*.

Following this experiment, carvacrol, ascorbic acid and tetrasodium pyrophosphate were selected to test the irradiation sensitivity of *E. coli* and *S. typhi* in the presence of different mixtures of additives. The best combinations of additives for both bacteria were carvacrol alone and the mixture of carvacrol and tetrasodium pyrophosphate. When these additives were added to ground beef samples, the irradiation sensitivity of both bacteria increased by more than 50 % compared to ground beef without additives.

When ground beef was mixed with the mixture of carvacrol and tetrasodium pyrophospate and packed under different atmospheres, the best results were obtained under MAP. Under these conditions, the irradiation sensitivity of *E. coli* was increased by 46.5 % and by 76.0 % for *S. typhi* when compared with samples packed under air without additives.

Finally, the irradiation sensitivity of *E. coli* and *S. typhi* was also affected by the irradiation temperature. When samples were frozen at –80°C, *E. coli* and *S. typhi* were more resistant to irradiation, even in presence of the mixture of carvacrol and tetrasodium pyrophosphate.

The best combination of additives, atmosphere and irradiation to radiosensitize both bacteria was samples containing the mixture of carvacrol and tetrasodium pyrophosphate packed under MAP. Also, with that combination, the TBARS content was the lowest. This means that not only did this combination of treatment helped to reduce the bacterial population with very small irradiation dose, but it also helped to reduce the lipid oxidation generated by irradiation.

Following these experiments, it would be interesting to continue the determination of irradiation sensitivity in the presence of the various additives tested with other bacteria, such as *Listeria, S. typhimurium, Bacillus cereus,* etc. In addition, the shelf life of the ground beef should be evaluated under the best conditions, as determined in these experiments.

7. Acknowledgements

I would like to thank :

Dr. Monique Lacroix, my master's research director, for allowing me to work on this project and for all the advice she has given me.

MDS Nordion for their financial and intellectual contributions to this project.

Dr Blaise Ouattara, for all the help with my reports and for the laughters.

All the personnel at MDS Nordion, for their time every morning.

All the other students in the laboratory for their help and advice.

8. References

Abbe, T. , Krockel, L. and Hill, C. 1995. Bacteriocins : modes of action and potentials in food preservation and control of food poisoning. Internal. J. food Microbiol. 28 : 169 – 185.

Adams, M. R. and Moss, M. O. 1995. Food Microbiology. The Royal society of chemistry. Cambridge. Pages 164 – 168, 181 – 210.

Agriculture et Agroalimentaire Canada. 1987. L'irradiation des aliments. Centre d'édition du gouvernement du Canada. Ottawa. 67 pages.

Agriculture et Agroalimentaire Canada. 2001. À propos de l'industrie canadienne de la viande rouge. http://www.agr.ca/cb/factsheets/2red_f.html

Anonymous. 2000. La crise de la bactérie *E. coli*. L'abattoir compte irradier son bœuf. J. de Québec. 27 juin 2000, page 9.

Beuchat, L. R. 1976. Sensitivity of *Vibrio parahaemolyticus* to spices and organic acids. J. Food Sci. 41 : 899 – 902.

Beuchat, L.R. and Golden, D.A. 1989. Antimicrobials occurring naturally in foods. Food Technol. January 1989. 134 – 142.

Borgstrom, G. 1968. Principles of food science. Volume 1 : Food technology. The Macmillan Company, London. Pages 25 –51.

Bourgeois, C. M., Mescle, J. F. and Zucca, J. 1996. Microbiologie alimentaire. Tome 1. Aspect microbiologique de la sécurité et de la qualité des aliments. Collection Sciences & techniques agroalimentaires. New York. Pages 89 – 99, 209 – 215, 332 – 345.

Boziaris, I. S. and Adams, M. R. 1999. Effect of chelators and nisin produced in situ on inhibition and inactivation of Gram negatives. Intern. J. Food Microbiol. 53 : 105 – 113.

Branen, A. L. and Davidson, P.M. 1983. Antimicrobials in foods. Marcel Dekker, Inc. New York. Page 63.

Buchanan, R. L. and Doyle, M. P. 1997. Foodborne disease significance of *Escherichia coli* O157 : H7 and other enterohemorrhagic *E. coli*. Food technol. 51 (10) : 69 – 75.

Buchanan, R. L. and Shepherd, A. J. 1981. Inhibition of *Aspergillus parasiticus* by thymol. J. Food Sci. 46 : 976 – 977.

Bullerman, L. B., Lieu, F. Y. and Seier, S. A. 1977. Inhibition of growth and afltoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J. Food Sci. 42 (4) : 1107 – 1109.

Caillet, S. 1998. Valorisation des lies levuriennes de chardonnay en vinification champenoise : Aspects physico-chimique et enzymatique de la décoloration. Thèse de Doctorat. Université de Reims Champagne-Ardenne. Pages 43 – 51.

Centre for Disease Control. 2000a. http://www.cdc.gov/foodnet/pub/iceid/2000/bender_j.htm

Centre for Disease Control. 2000b.
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_t.htm

Centre for Disease Control. 1999. Food-related illness and death in the United States.
http://cdc.gov/ncidod/eid/vol5no5/mead.ht,

CFIA. http://www.inspection.gc.ca/english/toc/agene.shtml

Chen, C.-C., Pearson, A. M., Gray, J. I. and Merkel, R. A. 1984. Effects of salt and some antioxidants upon the TBA numbers of meat. Food Chem. 14 : 167 – 172.

Chung, K.-T., Dickson, J. S. and Crouse, J. D. 1989. Effects of nisin on the growth of bacteria attached to meat. Appl. Env. Microbiol. 55 (6) : 1329 – 1333.

Chung, K.-T., Lu, Z. and Chou, M. W. 1998. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. Food Chem. Toxicol. 36 : 1053 – 1060.

Conner, D. E. 1993. Antimicrobial in food, Naturally occurring compounds. Marcel Dekker Inc. New York. Pages 441 – 468.

Costilow, R. N., Batshon, B. A., Bratzler, L. J. and Robach, D. A. 1955. Interaction between ascorbic acid and psychrophilic bacteria associated with the discoloration of prepackaged beef. Food Technol. 9 : 560 – 563.

Cutter, C. N. and Siragusa, G. R. 1994. Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. Food Microbiol. 11 : 481 – 489.

Decker, E. A. and Faraji, H. 1990. Inhibition of lipid oxidation by carnosine. JAOCS. 67 (10) : 650 – 652.

Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. Food Technol. 44 (11) : 100 – 112, 117.

Dempster, J .F., hawrysh, Z. J., Shand, P., Lahola-Chomiak, L. and Corletto, L. 1985. Effect of low-dose irradiation (radurization) on the shelf life of beefburgers stored at 3°C. J Food Technol. 20 : 145 – 154.

de Roos, K. B. 1997. How lipids influence food flavor. Food Technol. 51 (1) : 60 – 62.

Doyle, E. 1999. Food Irradiation. http://wisc.edu/fri/foodirrd.htm

Ellinger, R. H. 1972. Phosphates as food ingredients. CRC Press. Cleveland. Pages 9 – 10, 97 – 124.

Farag, R. S., Daw, Z. Y., Hewedi, F. M. and El-Baroty, G. S. A. 1989. Antimicrobial activity of some egyptian spice essential oils. J. Food Prot. 52 (9) : 665 – 667.

Farber, J.M. 1991. Microbiological aspects of modified-atmosphere packaging technology : A review. J. Food Prot. 54 (1) : 58 – 70.

Farbood, M. I., MacNeil, J. H. and Ostovar, K. 1976. Effect of rosemary spice extractive on growth of microorganisms in meats. J. Milk Food Technol. 39 (10) : 675 – 679.

Farkas, J., Andrassy, É and Horti, K. 1992. Combined effects of physical treatments and sporostatic factors on *Clostridium sporogenes* spores. II. Combined effects of gamma radiation, heat treatment, reduced a_w and reduced pH in canned luncheon meat. Acta Alimentaria. 21 (1) : 49 – 66.

Fisher, J.R., Fletcher, D.L., Cox, N.A. and Bailey, J.S. 1985. Microbiological properties of hard-cooked eggs in a citric acid-based preservative solution. J. Food Prot. 48 : 252.

Fisher, N. and Widder, S. 1997. How proteins influence food flavor. Food Technol. 51 (1) : 68 – 70.

FDA. 1997. Irradiation in the production, processing and handling of food. http://www.food-irradiation.com/meatdec3.htm

Frazier, W.C. 1967. Food Microbiology. McGraw-Hill Book Company. Toronto. Pages 252 – 182.

FSIS. 2001. Focus on ground beef. http://www.fsis.usda.gov/OA/pubs/focusgb.htm

Gamage, S. D., Faith, N. G., Luchansky, J. B., Buege, D. R. and Ingham, S. C. 1997. Inhibition of microbial growth in chub-packed ground beef by refrigeration (2°C) and medium-dose (2.2 to 2.4 kGy) irradiation. Internal. J. Food Microbiol. 37 : 175 – 182.

Giroux, M. and Lacroix, M. 1998. Nutritional adequacy of irradiated meat – A review. Food Res. Internal. 31 (4) : 257 – 264.

Giroux, M. 2000. Effet de la combinaison de traitements antioxydants et antimicrobiens sur la qualité microbiologique, biochimique et organoleptique du bœuf haché irradié. Thèse. INRS - Institut Armand-Frappier.

Giroux, M., Ouattara, B., Yefsah, R., Smoragiewicz, V., Saucier, L., and Lacroix, M. 2001. Combined effect of ascorbic acid and gamma irradiation on microbial and sensorial characteristics of beef patties during refrigerated storage. J. Agric. Food Chem. 49 : 919-925.

Gürsel, B. and Gürakan, G.C. 1997. Effects of gamma irradiation on the survival of *Listeria monocytogenes* and on its growth at refrigeration temperature in poultry and red meat. Poultry Sci. 76 : 1661 – 1664.

Harris, L. J., Fleming, H. P. and Klaenhammer, T. R. 1992. Developments in nisin research. Food Res. Internal. 25 : 57 – 66.

Hasting, J. W., Holzapfel, W. H. and Niemand, J. G. 1986. Radiation resistance of lactobacilli isolated from radurized meat relative to growth and environment. Applied Env. Microbiol. 52 (4) : 898 – 901.

Helander, I. M., Alakomi, H.-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E., Gorris, L. G. M. and von Wright, A. 1998. Characterization of the action of selected essential oil components on gram-negative bacteria. J. Agric. Food Chem. 46 : 3590 – 3595.

Hintlian, C. B. and Hotchkiss, J. H. 1986. The safety of modified atmosphere packaging: A review. Food Technol. 40(12) : 70 – 76.

ICMSF. 1980. Microbial ecology of foods, Volume 1 : Factors affecting life and death of microorganisms. Academic Press. Toronto. Pages 46 – 69.

Johnson, L. P., Williams, S" E., Neel, S. W. and Reagan, J. O. 1994. Foodservice industry market profile study : Nutritional and objective textural profile of food service ground beef. J. Animal Sci. 72 : 1487.

Jung, D.-S., Bodyfelt, F. W. and Daeschel, M. A. 1992. Influence of fat and emulsifiers on the efficacy of nisin in inhibiting *Listeria monocytogenes* in fluid milk. J. Dairy Sci. 75 : 387 – 393.

Juven, B. J., Kanner, J., Schved, F. and Weisslowicz, H. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J. Appl. Bacteriol. 76 : 626 – 631.

Kabara, J. J. 1981. Food-grade chemicals for use in designing food preservative systems. J. Food Prot. 44 (8) : 633 – 647.

Kalt, W., McDonald, J.E. and Donner, H. 2000. Anthocyanins, phenolics and antioxidant capacity of processed lowbush blueberry products. J. Food Sci. 65 (3) : 390 – 393.

Karapinar, M. and Aktug, S. E. 1987. Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole. Internal. J. Food Microbiol. 4 : 161 – 166.

Khan, N. S. and Hadi, S. M. 1998. Structural features of tannic acid important for DNA degradation in the presence of Cu(II). Mutagenesis. 13 (3) : 217 - 274.

Kim, J., Marshall, M. R. and Wei, C.-i. 1995a. Antibacterial activity of some essential oil components against five foodborne pathogens. J. Agric. Food Chem. 43 (11) : 2839 – 2845.

Kim, J., Marshall, M. R., Carnell, J.A., Preston III, J.F. and Wei, C.-i. 1995b. Antibacterial activity of carvacrol, citral and geraniol against *Salmonella typhimurium* in culture medium and on fish cubes. J. Food Sci. 60 (6) : 1364 – 1368, 1374.

Lacroix, M., Smoragiewicz, W., Pazdernik, L., Koné, M.I. and Krzystyniak, K. 1997. Prevention of lipid radiolysis by natural antioxidants from rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.). Food Res. Internal. 30 (6) : 457 – 462.

Lacroix, M., Mahrour, A. Beaulieu, M., Jobin, M., Nketsa-Tabiri, J. and Gagnon, M. 1998. Effect of the rate and dose rate of irradiation on the quality of mushrooms, shrimps and marinated poultry. Internal. Atomic Energy Agency. Vienna. 41 –51.

Lacroix, M. and Ouattara, B. 2000. Combined industrial processes with irradiation to assure innocuity and preservation of food products – a review. Food Res. Internal. 0 : 1 – 6.

Lambert, J.D. and Maxcy, R.B. 1984. Effect of gamma radiation on *Campylobacter jejuni.* J. Food Sci. 49 : 665 – 665 – 667.

Lambert, A. D., Smith, J. P. and Dodds, K. L. 1991a. Shelf life extension and microbiological safety of fresh meat – A review. Food Microbiol. 8 (4) : 267 – 297.

Lambert, A. D., Smith, J. P. and Dodds, K. L. 1991b. Combined effect of modified atmosphere packaging and low-dose irradiation on toxin production by *Clostridium botulinum* in fresh pork. J. Food Prot. 54 (2) : 94 – 101.

Lawlor, J. B., Sheehy, P.J.A., Kerry, J.P., Buckley, D.J. and Morrissey, P.A. 2000. Measuring oxidative stability of beef muscles obtained from animals supplemented with vitamin E using conventional and derivative spectrophotometry. J. Food Sci. 65 (6) : 1138 – 1141.

Lee, B. J., Hendricks, D. G. and Cornforth, D. P. 1998. Antioxidant effects of carnosine and phytic acid in a model beef system. J. Food Sci. 63 (3) : 394 – 198.

Lee, B. J., Hendricks, D. G. and Cornforth, D. P. 1999a. A comparison of carnosine and ascorbic acid on color and lipid stability in a ground beef patties model system. Meat Sci. 51 : 245 – 253.

Lee, J.-W., Yook, H.-S., Kim, S.-A., Lee, K.-H. and Byun, M.-W. 1999b. Effects of antioxidants and gamma irradiation on the shelf life of beef patties. J. Food Prot. 62 (6) : 619 – 624.

Lefebvre, N., Thibault, C. and Charbonneau, R. 1992. Improvement of shelf-life and wholesomeness of ground beef by irradiation 1. Microbial aspects. Meat Sci. 32 : 203 – 213.

Mahrour, A., Lacroix, M., Nketsa-Tabiri, J., Calderon, N. and Gagnon, M. 1998. Antioxidant properties of natural substances in irradiated fresh poultry. Radiat. Phys. Chem. 52 : 77 – 80.

Maxcy, R. B. 1982. Irradiation of food for public health protection. J. Food Prot. 45 (4) : 363 – 366.

MDS Nordion. 2001. Approved foods. http://www.mds.nordion/display_page.asp

Mermelstein, N. H. 1993. Controlling *E. coli* O157 : H7. Food Technol. 47 : 90 – 91.

Molins, R. A., Kraft, A. A., Olsen, D. G. and Hotcnkiss, D. K. 1984. Recovery of selected bacteria in media containing 0.5 % food grade poly- and pyrophosphate. J. Food Sci. 49 : 948 - 949.

Molins, R. A., Kraft, A. A. and Olsen, D. G. 1985. Effect of phosphate an bacterial growth in refrigerated uncooked Bratwurst. J. Food Sci., 50 : 531 – 532.

Murano, E. A. 1995. Irradiation of fresh meat. Food technol. 49 : 52 – 54.

Murdoch, D.I., 1950. Inhibitory action of citric acid on tomato juice flat-sour organisms. Food Res. 15 : 107.

Murphy, A., Kerry, J. P., Buckley, J. and Gray, I. 1998. The antioxidative properties of rosemary oleoresin and inhibition of off-flavours in precooked roast beef slices. J. Sci. Food Agric. 77 : 235 – 243.

Nettles, C. G. and Berafoot, S. F. 1993. Biochemical and genetic characteristics and bacteriocins of food-associated lactic acid bacteria. J. Food Prot. 56 (4) : 338 – 356.

OMS. 1995. Les produits alimentaires ionisés : salubrité et valeur nutritive. Strategic Communications. Genève. Pages 39 – 51.

Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J.-P. and Bégin, A. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Internal. J. Food Micrbiol. 37 : 155 – 162.

Ouattara, B., Giroux, M., Smoragiewicz, W., Saucier, L. and Lacroix, M. 2002. Combined effect of gamma irradiation, ascorbic acid, and edible coating on the improvement of microbial and biochemical characteristics of ground beef. J. Food. Sci. 65 (6) : 981 - 987.

Paster, N., Menasherov, M. Ravid, U. and Juven, B. 1995. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J. Food Prot. 58 (1) : 81 – 85.

Patterson, M. 1988. Sensitivity of bacteria to irradiation on poultry meat under various atmospheres. Lett. Appl. Microbiol. 7 : 55 – 58.

Pelmont, J. 1993. Bactéries et environnement. Presse Université de Grenoble. Grenoble. 178 – 180.

Powers, J.J., Somaatmadja, D., Pratt, D.E. and Hamdy, M.K. 1960. Anthocyanins. II. Action of anthocyanin pigments and related compounds on the growth of certain microoganisms. Food Technol. 14 : 626.

Radomyshi, T., Murano, E. A., Olson, D. G. and Murano, P.S. 1994. Elimination of pathogens of significance in food by low-dose irradiation : a review. J. Food Prot. 57 (1) : 73 – 86.

Rice-Evans, C. 2001. Flavonoid antioxidants. Current Med. Chem. 8 : 797 – 807.

Roberts, W. T. and Weese, J. O. 1998. Shelf life of ground beef patties treated by gamma radiation. J. Food Prot. 61 (10) : 1387 – 1389.

Saha, U., Murthy, T. R. K. and Kowale, B. N. 1999. Effect of EDTA and ascorbic acid dip treatment on storage stability of goat meat at refrigeration temperature. J. Food Sci. Technol. 36 (2) : 180 – 183.

Santé Canada. 2002. Loi sur les aliments et drogues. www.hc-sc.gc.ca

Santé Canada. 2003. Rapport mensuel des maladies à déclaration obligatoire. www.hc-sc.gc.ca

Schaefer, D. M., Liu, Q., Faustman, C. and Yin, M.-c. 1995. Supranutritional administration of vitamins E and C improves oxidative stability of beef. J. Nutrition. 125 (6) : 1792S – 1798S.

Sebranek, J. 1999. Pro-oxidants and antioxidants. Meat Proc. 96.

Shelef, L. A., Naglik, O. A. and Bogen, D. W. 1980. Sensitivity of some common food-borne bacteria to the spices sage, rosemary and allspice. J. Food Sci. 45 (4) : 1042 – 1044.

Simard, R.E. 1995. Innocuité alimentaire : Analyse du risque, cas du Canada. C.R. Acad Agric. Fr. 81 (7) : 129 - 140.

Siragusa, G. R., Cutter, C. N. and Willett, J. L. 1999. Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. Food Microbiol. 16 : 229 – 235.

Snyder, O.P. 1997. Antimicrobial effects of spices and herbs. http://hi-tm.com/documents/spices.html

Sofos, J.N. and Busta, F.F. 1981. Antimicrobial activity of sorbate. J. Food Prot. 44(8) : 614 – 622.

Statistique Canada. 2000. Consommation de la viande rouge. Le Quotidien. http://statcan.ca/daily/francais/000503/q000503g.htm

Stecchinni, M. L., Del Torre, M., Sarais, I., Fuochi, P.G., Tubaro, F. and Ursini, F. 1998. Carnosine increases irradiation resistance of *Aeromonas hydrophila* in minced turkey meat. J. Food Sci. 63 (1) : 147 – 149.

Stevens, K. A., Sheldon, B. W., Klapes, N. A. and Klaenhammer. 1991. Nisin treatment for inactivation of *Salmonella* species and other gram-vegetative bacteria. Appl. Env. Microbiol. 57 (12) : 3613 – 3615.

Stevens, K. A., Sheldon, B. W., Klapes, N. A. and Klaenhammer. 1992. Effect of treatment conditions on nisin inactivation of gram-negative bacteria. J. Food Prot. 55 (10) : 763 – 766.

Suresh, P. Ingle, V.K., and Vijayalakshmi, V. 1992. Antibacterial activity of eugenol in comparison with other antibiotics. J. Food Sci. Technol. 29: 254-256.

Thakur, B.R. and Singh, R.K. 1994. Food irradiation – Chemistry and applications. Food Res. Internal. 10 (4) : 437 – 473.

Thayer, D. W. 1993. Extending shelf life of poultry and red meat by irradiation processing. J. Food Prot. 56 (10) : 831 – 833, 846.

Thayer, D. W. and Boyd, G. 1999. Irradiation and modified atmosphere packaging for the control of *Listeria monocytogenes* on turkey meat. J. Food Prot. 62 (10) : 1136 – 1142.

Thayer, D. W., Songprasertchai, S. and Boyd, G. 1991. Effects of heat and ionizing radiation on *Salmonella typhimurium* in mechanically deboned chicken meat. J. Food Prot. 54 : 718 – 724.

Thompson, J.E., Banwart, G.J., Sanders, D.H. and Mercuri, A.J. 1967. Effect of chlorine, antibiotics , β-propiolactone, acids and washing on *Salmonella typhimurium* on eviscerated fryer chickens. Poultry Sci. 46 : 146.

Triebold, H. O. and Aurand, L. W. 1963. Food composition and analysis. D. Van Nostrand Company, Inc. Toronto. Pages 362 – 375.

Ultee, A. Lets, E. P. W. and Smid, E. J. 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl. Env. Microbiol. 65 (10) : 4606 – 4610.

Ultee, A. Slump, R. A., Steging, G. and Smid, E. J. 2000. Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. J. Food Prot. 63 (5) : 620 – 624.

Université Laval. 1997. Note de cours : Aliments et conservation. Section 1, module 2. Page 26 – 27.

USDA. 2001a. http://usda.mannlib.cornell.edu

USDA. 2001b. http://usda.mannlib.cornell.edu/reports/nassr/livestock/pls-bban/isan0301.txt

von Sonntag, C. 1987. The chemical basis of radiation biology. Taylor & Francis, New York. 505 pages.

Welbourn, Jerry L. 1998. Campylobacter : no longer the "quiet pathogen". Scope. 13 (2) : 1 - 4.

Woolford, M.K. 1975. Microbiological screening of food preservatives, cold sterilants and specific antimicrobial agents as potential silage additives. J. Sci. Food. Agric. 26 : 229.

Zamora, M.C. and Zaritzky, N.E. 1987. Antimicrobial activity of undissociated sorbic acid in vacuum packaged beef. J. Food Sci. 52(6) : 1449 – 1454.

Zheng, W. and Wang, S.Y. 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food. Chem. 49 : 5165 – 5170.

9. Annex

Table 19 : Bacterial count (log CFU/g) of *E. coli* in ground beef in the presence of various concentrations of additives for the determination of MIC value

Concentrations	Ascorbic acid	Tannic acid	Carvacrol	Thyme	Thymol	Trans-cinnamaldehyde	Rosemary
0,00	6,13 ± 0,27	5,73 ± 0,04	5,69 ± 0,03	6,28 ± 0,24	6,29 ± 0,37	6,08± 0,10	5,77 ± 0,13
0,50	5,83 ± 0,08	5,74 ± 0,04	5,59 ± 0,07	6,14 ± 0,31	5,86 ± 0,10	5,38 ± 0,06	
1,00	5,92 ± 0,04	5,67 ± 0,06	5,45 ± 0,20	6,01 ± 0,24	5,59 ± 0,16	5,50 ± 0,25	5,74 ± 0,24
1,50	5,55 ± 0,22	5,70 ± 0,10	4,74 ± 0,12	5,80 ± 0,03	5,47 ± 0,15	5,23 ± 0,11	
2,00	5,45 ± 0,13	5,62 ± 0,14	3,94 ± 0,12	5,94 ± 0,46	4,92 ± 0,17	5,01 ± 0,11	5,58 ± 0,15
2,50	5,19 ± 0,06	5,50 ± 2,00e-3	2,67 ± 0,00	5,58 ± 0,57	4,14 ± 0,37	4,41 ± 0,17	
3,00	5,07 ± 0,10	5,47 ± 0,02		4,85 ± 0,28	3,44 ± 0,10	3,88 ± 0,11	5,30 ± 0,28

	Duralox AR Seasoning MDF	Duralox Oxidation NMC-2	Duralox Oxidation NC-2 type C	Herbalox Type HTO	Herbalox Type HT-25	Herbalox Type o
0,00	5,55 ± 0,03	5,55 ± 0,03	5,55 ± 0,03	5,55 ± 0,03	5,55 ± 0,03	5,55 ± 0,03
0,20	5,50 ± 0,13	5,48 ± 0,07	5,55 ± 0,05	5,64 ± 0,05	5,60 ± 0,09	5,65 ± 0,03
0,50	5,30 ± 0,08	5,41 ± 0,12	5,43 ± 1,94e-3	5,38 ± 0,08	5,46 ± 0,01	5,67 ± 0,03
1,50	5,07 ± 0,11	5,18 ± 0,25	5,13 ± 0,03	5,32 ± 0,03	5,12 ± 0,10	5,38 ± 0,10
2,50	4,86 ± 0,02	5,14 ± 0,01	5,23 ± 0,05	5,30 ± 0,06	5,17 ± 0,04	5,38 ± 0,06

Tableau 19a : Compte bactérien (log UFC/g) de *E. coli* dans le bœuf haché en présence de différentes concentrations d'additifs pour la détermination du MIC

Concentrations	Acide ascorbique	Acide tannique	Carvacrol	Thym	Thymol	Trans-cinnamaldehyde	Romarin
0,00	6,13 ± 0,27	5,73 ± 0,04	5,69 ± 0,03	6,28 ± 0,24	6,29 ± 0,37	6,08± 0,10	5,77 ± 0,13
0,50	5,83 ± 0,08	5,74 ± 0,04	5,59 ± 0,07	6,14 ± 0,31	5,86 ± 0,10	5,38 ± 0,06	
1,00	5,92 ± 0,04	5,67 ± 0,06	5,45 ± 0,20	6,01 ± 0,24	5,59 ± 0,16	5,50 ± 0,25	5,74 ± 0,24
1,50	5,55 ± 0,22	5,70 ± 0,10	4,74 ± 0,12	5,80 ± 0,03	5,47 ± 0,15	5,23 ± 0,11	
2,00	5,45 ± 0,13	5,62 ± 0,14	3,94 ± 0,12	5,94 ± 0,46	4,92 ± 0,17	5,01 ± 0,11	5,58 ± 0,15
2,50	5,19 ± 0,06	5,50 ± 2,00e-3	2,67 ± 0,00	5,58 ± 0,57	4,14 ± 0,37	4,41 ± 0,17	
3,00	5,07 ± 0,10	5,47 ± 0,02		4,85 ± 0,28	3,44 ± 0,10	3,88 ± 0,11	5,30 ± 0,28

	Duralox AR Seasoning MDF	Duralox Oxidation NMC-2	Duralox Oxidation NC-2 type C	Herbalox Type HTO	Herbalox Type HT-25	Herbalox Type o
0,00	5,55 ± 0,03	5,55 ± 0,03	5,55 ± 0,03	5,55 ± 0,03	5,55 ± 0,03	5,55 ± 0,03
0,20	5,50 ± 0,13	5,48 ± 0,07	5,55 ± 0,05	5,64 ± 0,05	5,60 ± 0,09	5,65 ± 0,03
0,50	5,30 ± 0,08	5,41 ± 0,12	5,43 ± 1,94e-3	5,38 ± 0,08	5,46 ± 0,01	5,67 ± 0,03
1,50	5,07 ± 0,11	5,18 ± 0,25	5,13 ± 0,03	5,32 ± 0,03	5,12 ± 0,10	5,38 ± 0,10
2,50	4,86 ± 0,02	5,14 ± 0,01	5,23 ± 0,05	5,30 ± 0,06	5,17 ± 0,04	5,38 ± 0,06

Table 20 : Bacterial count (log CFU/g) of *S. typhi* in ground beef in the presence of various concentrations of additives for the determination of MIC value

Concentration	Ascorbic acid	Tannic acid	Carvacrol	Thyme	Thymol	Trans-cinnamaldehyde	rosemary
0,00	6.06 ± 0.06	5.10 ± 0.02	5.37 ± 0.07	5.73 ± 0.00	5.32 ± 0.05	5.35 ± 0.15	5.38 ± 0.06
0,50			5.06 ± 0.04	5.58 ± 0.01	5.11 ± 0.09	4.66 ± 0.05	
1,00	5.73 ± 0.09	5.08 ± 0.07	4.41 ± 0.01	5.35 ± 0.06	5.10 ± 0.03	4.11 ± 0.05	5.70 ± 0.01
1,50			4.08 ± 0.04	5.28 ± 0.05	4.60 ± 0.02	3.23 ± 0.03	
2,00	5.22 ± 0.07	5.07 ± 0.01	3.39 ± 0.10	5.15 ± 0.04	3.65 ± 0.09	2.96 ± 0.08	5.26 ± 0.02
2,50			3.66 ± 0.01	4.89 ± 0.04	2.39 ± 0.12	2.47 ± 0.00	
3,00	4.42 ± 0.05	5.06 ± 0.05	2.56 ± 0.05	4.61 ± 0.04	0.00 ± 0.00	1.97 ± 0.01	5.27 ± 0.01
4.00		4.92 ± 0.07					4.71 ± 0.00
5.00		4.88 ± 0.05					

	Duralox Oxidation NC-2 type C	Duralox AR Seasoning MDF	Duralox Oxidation NMC-2	Herbalox Type o	Herbalox Type HT-25	Herbalox Type HTO
0,00	5,20 ± 0,03	5,20 ± 0,03	5,20 ± 0,03	5,20 ± 0,03	5,20 ± 0,03	5,20 ± 0,03
2,00	5,22 ± 0,03	5,18 ± 0,05	4,97 ± 0,05	5,02 ± 0,08	5,09 ± 0,04	5,01 ± 0,11
4,00	5,14 ± 0,05	5,12 ± 0,04	5,09 ± 0,03	5,02 ± 0,03	5,06 ± 0,08	4,97 ± 0,08
6,00	5,13 ± 0,03	5,18 ± 0,07	5,06 ± 0,07	5,08 ± 0,11	5,08 ± 0,02	5,02 ± 0,05
8,00	5,08 ± 0,03	5,15 ± 0,06	5,05 ± 0,05	5,08 ± 0,10	4,98 ± 0,08	4,98 ± 0,08
10,00	5,05 ± 0,07	5,18 ± 3,86e-3	5,05 ± 0,05	5,04 ± 0,03	4,96 ± 0,06	4,98 ± 0,06

Tableau 20a: Compte bactérien (log UFC/g) de *S. typhi* dans le bœuf haché en présence de différentes concentrations d'additifs pour la détermination du MIC

Concentration	Ascorbic acid	Tannic acid	Carvacrol	Thyme	Thymol	Trans-cinnamaldehyde	rosemary
0,00	6.06 ± 0.06	5.10 ± 0.02	5.37 ± 0.07	5.73 ± 0.00	5.32 ± 0.05	5.35 ± 0.15	5.38 ± 0.06
0,50			5.06 ± 0.04	5.58 ± 0.01	5.11 ± 0.09	4.66 ± 0.05	
1,00	5.73 ± 0.09	5.08 ± 0.07	4.41 ± 0.01	5.35 ± 0.06	5.10 ± 0.03	4.11 ± 0.05	5.70 ± 0.01
1,50			4.08 ± 0.04	5.28 ± 0.05	4.60 ± 0.02	3.23 ± 0.03	
2,00	5.22 ± 0.07	5.07 ± 0.01	3.39 ± 0.10	5.15 ± 0.04	3.65 ± 0.09	2.96 ± 0.08	5.26 ± 0.02
2,50			3.66 ± 0.01	4.89 ± 0.04	2.39 ± 0.12	2.47 ± 0.00	
3,00	4.42 ± 0.05	5.06 ± 0.05	2.56 ± 0.05	4.61 ± 0.04	0.00 ± 0.00	1.97 ± 0.01	5.27 ± 0.01
4.00		4.92 ± 0.07					4.71 ± 0.00
5.00		4.88 ± 0.05					

	Duralox Oxidation NC-2 type C	Duralox AR Seasoning MDF	Duralox Oxidation NMC-2	Herbalox Type o	Herbalox Type HT-25	Herbalox Type HTO
0,00	5,20 ± 0,03	5,20 ± 0,03	5,20 ± 0,03	5,20 ± 0,03	5,20 ± 0,03	5,20 ± 0,03
2,00	5,22 ± 0,03	5,18 ± 0,05	4,97 ± 0,05	5,02 ± 0,08	5,09 ± 0,04	5,01 ± 0,11
4,00	5,14 ± 0,05	5,12 ± 0,04	5,09 ± 0,03	5,02 ± 0,03	5,06 ± 0,08	4,97 ± 0,08
6,00	5,13 ± 0,03	5,18 ± 0,07	5,06 ± 0,07	5,08 ± 0,11	5,08 ± 0,02	5,02 ± 0,05
8,00	5,08 ± 0,03	5,15 ± 0,06	5,05 ± 0,05	5,08 ± 0,10	4,98 ± 0,08	4,98 ± 0,08
10,00	5,05 ± 0,07	5,18 ± 3,86e-3	5,05 ± 0,05	5,04 ± 0,03	4,96 ± 0,06	4,98 ± 0,06

Table 21 : Normalised bacterial count (log CFU/g) of *E. coli* in ground beef in the presence of various additives at various irradiation doses for the determination of D_{10} value

Irradiation doses	Control	Ascorbic acid (0.5%)	Tannic acid (0.38%)	BHA (0.01%)	BHT (0.01%)	Carnosine (1.0%)	Carvacrol (0.125%)
0.00	5.180 ± 0.145	5.170 ± 0.050	5.174 ± 0.047	5.170 ± 0.038	5.172 ± 0.076	5.171 ± 0.062	5.179 ± 0.077
0.25	3.171 ± 0.206	3.479 ± 0.214	2.463 ± 0.171	2.716 ± 0.161	2.891 ± 0.114	3.306 ± 0.108	2.724 ± 0.090
0.35	2.274 ± 0.332	2.956 ± 0.091	1.678 ± 0.411	1.703 ± 0.440	1.976 ± 0.153	2.435 ± 0.091	1.972 ± 0.261
0.45	1.404 ± 0.486	2.007 ± 0.191	0.725 ± 0.085	0.877 ± 0.253	1.373 ± 0.276	1.633 ± 0.101	0.893 ± 0.174
0.55	0.771 ± 0.334	1.518 ± 0.357	0.000 ± 0.000	0.632 ± 0.086	0.301 ± 0.280	1.152 ± 0.286	0.560 ± 0.164

Irradiation doses	Carvacrol (0.88%)	EDTA (100 ppm)	Nisin (625 UI/g)	Nisin (625 UI/g) + EDTA (100 ppm)	Tetrasodium pyrophosphate (0.1%)	Rosemary (0.5%)	Thyme (0.2%)
0.00	5.181 ± 0.059	5.171 ± 0.044	5.170 ± 0.062	5.170 ± 0.089	5.170 ± 0.048	5.170 ± 0.056	5.170 ± 0.090
0.10	4.001 ± 0.107						
0.15	3.681 ± 0.200						
0.20	3.051 ± 0.157						
0.25	2.697 ± 0.097	3.224 ± 0.137	3.339 ± 0.399	3.099 ± 0.111	3.219 ± 0.098	3.082 ± 0.134	2.928 ± 0.080
0.30	2.254 ± 0.058						
0.35		2.191 ±0.091	2.274 ± 0.673	2.314 ± 0.256	2.741 ± 0.146	1.971 ± 0.181	2.125 ± 0.142
0.45		1.418 ± 0.299	1.526 ± 0.422	1.307 ± 0.175	1.535 ± 0.251	1.174 ± 0.111	1.031 ± 0.710
0.55		1.042 ± 0.177	0.584 ± 0.404	0.729 ± 0.301	1.086 ± 0.345	0.261 ± 0.231	0.560 ± 0.035

Irradiation doses	Thyme (2.33%)	Thymol (0.1%)	Thymol (1.15%)	Trans-cinnamaldehyde (0.025%)	Trans-cinnamaldehyde (1.5%)
0.00	5.170 ± 0.052	5.170 ± 0.045	5.181 ± 0.068	5.170 ± 0.068	5.182 ± 0.098
0.10			3.918 ± 0.142		1.767 ± 0.283
0.15			3.410 ± 0.141		0.372 ± 0.168
0.20			2.879 ± 0.163		0.000 ± 0.000
0.25	1.540 ± 0.466	2.450 ± 0.546	2.234 ± 0.122	2.722 ± 0.157	
0.30					
0.35	1.264 ± 0.490	2.088 ± 0.332		2.206 ± 0.320	
0.45	0.133 ± 0.279	0.743 ± 0.646		1.223 ± 0.223	
0.55		0.000 ± 0.000		0.335 ± 0.208	

Tableau 21a : Compte bactérien normalisé (log UFC/g) de *E. coli* dans le bœuf haché en présence de différents d'additifs à différentes doses d'irradiation pour la détermination de la D_{10}

Doses d'irradiation	Témoin	Acide ascorbique (0.5%)	Acide tannique (0.38%)	BHA (0.01%)	BHT (0.01%)	Carnosine (1.0%)	Carvacrol (0.125%)
0.00	5.180 ± 0.145	5.170 ± 0.050	5.174 ± 0.047	5.170 ± 0.038	5.172 ± 0.076	5.171 ± 0.062	5.179 ± 0.077
0.25	3.171 ± 0.206	3.479 ± 0.214	2.463 ± 0.171	2.716 ± 0.161	2.891 ± 0.114	3.306 ± 0.108	2.724 ± 0.090
0.35	2.274 ± 0.332	2.956 ± 0.091	1.678 ± 0.411	1.703 ± 0.440	1.976 ± 0.153	2.435 ± 0.091	1.972 ± 0.261
0.45	1.404 ± 0.486	2.007 ± 0.191	0.725 ± 0.085	0.877 ± 0.253	1.373 ± 0.276	1.633 ± 0.101	0.893 ± 0.174
0.55	0.771 ± 0.334	1.518 ± 0.357	0.000 ± 0.000	0.632 ± 0.086	0.301 ± 0.280	1.152 ± 0.286	0.560 ± 0.164

Doses d'irradiation	Carvacrol (0.88%)	EDTA (100 ppm)	Nisine (625 UI/g)	Nisine (625 UI/g) + EDTA (100 ppm)	Tétrasodium pyrophosphate (0.1%)	Romarin (0.5%)	Thym (0.2%)
0.00	5.181 ± 0.059	5.171 ± 0.044	5.170 ± 0.062	5.170 ± 0.089	5.170 ± 0.048	5.170 ± 0.056	5.170 ± 0.090
0.10	4.001 ± 0.107						
0.15	3.681 ± 0.200						
0.20	3.051 ± 0.157						
0.25	2.697 ± 0.097	3.224 ± 0.137	3.339 ± 0.399	3.099 ± 0.111	3.219 ± 0.098	3.082 ± 0.134	2.928 ± 0.080
0.30	2.254 ± 0.058						
0.35		2.191 ±0.091	2.274 ± 0.673	2.314 ± 0.256	2.741 ± 0.146	1.971 ± 0.181	2.125 ± 0.142
0.45		1.418 ± 0.299	1.526 ± 0.422	1.307 ± 0.175	1.535 ± 0.251	1.174 ± 0.111	1.031 ± 0.710
0.55		1.042 ± 0.177	0.584 ± 0.404	0.729 ± 0.301	1.086 ± 0.345	0.261 ± 0.231	0.560 ± 0.035

Doses d'irradiation	Thym (2.33%)	Thymol (0.1%)	Thymol (1.15%)	Trans-cinnamaldéhyde (0.025%)	Trans-cinnamaldéhyde (1.5%)
0.00	5.170 ± 0.052	5.170 ± 0.045	5.181 ± 0.068	5.170 ± 0.068	5.182 ± 0.098
0.10			3.918 ± 0.142		1.767 ± 0.283
0.15			3.410 ± 0.141		0.372 ± 0.168
0.20			2.879 ± 0.163		0.000 ± 0.000
0.25	1.540 ± 0.466	2.450 ± 0.546	2.234 ± 0.122	2.722 ± 0.157	
0.30					
0.35	1.264 ± 0.490	2.088 ± 0.332		2.206 ± 0.320	
0.45	0.133 ± 0.279	0.743 ± 0.646		1.223 ± 0.223	
0.55		0.000 ± 0.000		0.335 ± 0.208	

Table 22 : Normalised bacterial count (log CFU/g) of *S. typhi* in ground beef in the presence of various additives at various irradiation doses for the determination of D_{10} value

Irradiation doses	Control	Ascorbic acid (0.5%)	Tannic acid (0.38%)	BHA (0.01%)	BHT (0.01%)	Carnosine (1.0%)	Carvacrol (0.125%)
0.00	5.287 ± 0.187	5.170 ± 0.050	5.174 ± 0.047	5.170 ± 0.038	5.172 ± 0.076	5.171 ± 0.062	5.179 ± 0.077
0.25		5.031 ± 0.137	3.904 ± 0.436	4.499 ± 0.047	5.078 ± 0.254	4.574 ± 0.097	4.370 ± 0.176
0.50	4.529 ± 0.267	4.595 ± 0.273	2.926 ± 0.929	4.304 ± 0.142	4.582 ± 0.247	4.226 ± 0.221	4.032 ± 0.131
0.75	4.211 ± 0.154	4.100 ± 0.240	2.316 ± 0.584	2.835 ± 0.128	4.012 ± 0.158	3.679 ± 0.138	3.290 ± 0.200
1.00	3.852 ± 0.188	3.611 ± 0.102	1.336 ± 0.078	2.698 ± 0.146	3.236 ± 0.230	3.161 ± 0.074	2.268 ± 0.329
1.25		2.671 ± 0.209	1.245 ± 0.455	2.228 ± 0.101	2.081 ± 0.168	2.629 ± 0.172	1.558 ± 0.217
1.50	2.514 ± 0.100						
2.00	1.597 ± 0.069						

Irradiation doses	Carvacrol (1.15%)	EDTA (100 ppm)	Nisine (625 UI/g)	Nisine (625 UI/g) + EDTA (100 ppm)	Tetrasodium pyrophosphate (0.1%)	Rosemary (0.5%)	Thyme (0.2%)
0.00	5.181 ± 0.059	5.171 ± 0.044	5.170 ± 0.062	5.170 ± 0.089	5.170 ± 0.048	5.170 ± 0.056	5.170 ± 0.090
0.25	4.226 ± 0.270	4.637 ± 0.069	4.627 ± 0.284	4.664 ± 0.078	4.686 ± 0.088	4.547 ± 0.185	4.670 ± 0.100
0.50	3.352 ± 0.241	4.147 ± 0.099	4.308 ± 0.087	4.087 ± 0.135	4.200 ± 0.160	4.383 ± 0.197	4.100 ± 0.251
0.75	2.528 ± 0.404	3.823 ± 0307	3.598 ± 0157	3.391 ± 0.121	3.448 ± 0.104	3.484 ± 0.163	3.362 ± 0.222
1.00	1.117 ± 1.050	2.743 ± 0160	2.865 ± 0403	2.717 ± 0.312	2.705 ± 0.105	2.937 ± 0.111	2.652 ± 0.178
1.25		2.107 ± 0.233	1.761 ± 0.441	1.468 ± 0.443	1.710 ± 0.242	2.263 ± 0.142	2.095 ± 0.181

Irradiation doses	Thyme (2.75 %)	Thymol (0.1%)	Thymol (1.6%)	Trans-cinnamaldehyde (0.025%)	Trans-cinnamaldehyde (0.89%)
0.00	5.170 ± 0.052	5.170 ± 0.045	5.181 ± 0.068	5.170 ± 0.068	5.182 ± 0.098
0.25	4.369 ± 0.435	4.730 ± 0.132	4.445 ± 0.742	4.673 ± 0.141	3.840 ± 0.213
0.40					2.587 ± 0.099
0.50	3.734 ± 0.280	4.349 ± 0.134	3.633 ± 0.853	4.355 ± 0.442	1.304 ± 0.382
0.60					0.796 ± 0.030
0.75	3.310 ± 0.463	3.505 ± 0.164	2.497 ± 0.399	3.030 ± 0.092	0.000 ± 0.000
1.00	1.884 ± 0.967	2.593 ± 0.174	1.006 ± 0.663	2.590 ± 0.455	
1.25	0.705 ± 0.774	1.714 ± 0.214		1.895 ± 0.217	

Tableau 22a : Compte bactérien normalisé (log UFC/g) de *S. typhi* dans le bœuf haché en présence de différents d'additifs à différentes doses d'irradiation pour la détermination de la D_{10}

Doses d'irradiation	Témoin	Acide ascorbique (0.5%)	Acide tannique (0.38%)	BHA (0.01%)	BHT (0.01%)	Carnosine (1.0%)	Carvacrol (0.125%)
0.00	5.287 ± 0.187	5.170 ± 0.050	5.174 ± 0.047	5.170 ± 0.038	5.172 ± 0.076	5.171 ± 0.062	5.179 ± 0.077
0.25		5.031 ± 0.137	3.904 ± 0.436	4.499 ± 0.047	5.078 ± 0.254	4.574 ± 0.097	4.370 ± 0.176
0.50	4.529 ± 0.267	4.595 ± 0.273	2.926 ± 0.929	4.304 ± 0.142	4.582 ± 0.247	4.226 ± 0.221	4.032 ± 0.131
0.75	4.211 ± 0.154	4.100 ± 0.240	2.316 ± 0.584	2.835 ± 0.128	4.012 ± 0.158	3.679 ± 0.138	3.290 ± 0.200
1.00	3.852 ± 0.188	3.611 ± 0.102	1.336 ± 0.078	2.698 ± 0.146	3.236 ± 0.230	3.161 ± 0.074	2.268 ± 0.329
1.25		2.671 ± 0.209	1.245 ± 0.455	2.228 ± 0.101	2.081 ± 0.168	2.629 ± 0.172	1.558 ± 0.217
1.50	2.514 ± 0.100						
2.00	1.597 ± 0.069						

Doses d'irradiation	Carvacrol (1.15%)	EDTA (100 ppm)	Nisine (625 UI/g)	Nisine (625 UI/g) + EDTA (100 ppm)	Tétrasodium pyrophosphate (0.1%)	Romarin (0.5%)	Thyme (0.2%)
0.00	5.181 ± 0.059	5.171 ± 0.044	5.170 ± 0.062	5.170 ± 0.089	5.170 ± 0.048	5.170 ± 0.056	5.170 ± 0.090
0.25	4.226 ± 0.270	4.637 ± 0.069	4.627 ± 0.284	4.664 ± 0.078	4.686 ± 0.088	4.547 ± 0.185	4.670 ± 0.100
0.50	3.352 ± 0.241	4.147 ± 0.099	4.308 ± 0.087	4.087 ± 0.135	4.200 ± 0.160	4.383 ± 0.197	4.100 ± 0.251
0.75	2.528 ± 0.404	3.823 ± 0307	3.598 ± 0157	3.391 ± 0.121	3.448 ± 0.104	3.484 ± 0.163	3.362 ± 0.222
1.00	1.117 ± 1.050	2.743 ± 0160	2.865 ± 0403	2.717 ± 0.312	2.705 ± 0.105	2.937 ± 0.111	2.652 ± 0.178
1.25		2.107 ± 0.233	1.761 ± 0.441	1.468 ± 0.443	1.710 ± 0.242	2.263 ± 0.142	2.095 ± 0.181

Doses d'irradiation	Thym (2.75 %)	Thymol (0.1%)	Thymol (1.6%)	Trans-cinnamaldéhyde (0.025%)	Trans-cinnamaldéhyde (0.89%)
0.00	5.170 ± 0.052	5.170 ± 0.045	5.181 ± 0.068	5.170 ± 0.068	5.182 ± 0.098
0.25	4.369 ± 0.435	4.730 ± 0.132	4.445 ± 0.742	4.673 ± 0.141	3.840 ± 0.213
0.40					2.587 ± 0.099
0.50	3.734 ± 0.280	4.349 ± 0.134	3.633 ± 0.853	4.355 ± 0.442	1.304 ± 0.382
0.60					0.796 ± 0.030
0.75	3.310 ± 0.463	3.505 ± 0.164	2.497 ± 0.399	3.030 ± 0.092	0.000 ± 0.000
1.00	1.884 ± 0.967	2.593 ± 0.174	1.006 ± 0.663	2.590 ± 0.455	
1.25	0.705 ± 0.774	1.714 ± 0.214		1.895 ± 0.217	

Table 23 : Normalised bacterial count (log CFU/g) of *E. coli* in ground beef in the presence of various combinations of carvacrol (1.0 %), ascorbic acid (0.5 %) and tetrasodium pyrophosphate (0.1 %) at various irradiation doses for the determination of D_{10} value

Irradiation doses	Control	Carvacrol	Carvacrol + ascorbic acid	Carvacrol + tetrasodium pyrophosphate	Carvacrol + ascorbic acid + tetrasodium pyrophosphate
0.00	5.224 ± 0.098	5.224 ± 0.055	5.224 ± 0.026	5.224 ± 0.088	5.224 ± 0.101
0.10	4.629 ± 0.156	2.992 ± 0.263	4.640 ± 0.122	3.265 ± 0.293	4.549 ± 0.161
0.15		1.435 ± 0.698		2.267 ± 0.168	
0.20	3.722 ± 0.089	0.483 ± 0.685	3.709 ± 0.162	0.940 ± 0.285	3.665 ± 0.085
0.25		0.116 ± 0.195		0.450 ± 0.005	
0.30	3.095 ± 0.091	0.000 ± 0.000	2.930 ± 0.156	0.000 ± 0.000	3.000 ± 0.152
0.40	2.172 ± 0.164		2.589 ± 0.146		2.127 ± 0.151
0.50	1.361 ± 0.169		1.409 ± 0.159		1.741 ± 0.161
0.60	0.647 ± 0.205		0.751 ± 0.085		1.117 ± 0.086

Tableau 23a : Compte bactérien normalisé (log UFC/g) de *E. coli* dans le bœuf haché en présence de différentes combinaisons de carvacrol (1.0 %), d'acide ascorbique (0.5 %) et tétrasodium pyrophosphate (0.1 %) à différentes doses d'irradiation pour la détermination de la D_{10}

Doses d'irradiation	Témoin	Carvacrol	Carvacrol + acide ascorbique	Carvacrol + tétrasodium pyrophosphate	Carvacrol + acide ascorbique + tétrasodium pyrophosphate
0.00	5.224 ± 0.098	5.224 ± 0.055	5.224 ± 0.026	5.224 ± 0.088	5.224 ± 0.101
0.10	4.629 ± 0.156	2.992 ± 0.263	4.640 ± 0.122	3.265 ± 0.293	4.549 ± 0.161
0.15		1.435 ± 0.698		2.267 ± 0.168	
0.20	3.722 ± 0.089	0.483 ± 0.685	3.709 ± 0.162	0.940 ± 0.285	3.665 ± 0.085
0.25		0.116 ± 0.195		0.450 ± 0.005	
0.30	3.095 ± 0.091	0.000 ± 0.000	2.930 ± 0.156	0.000 ± 0.000	3.000 ± 0.152
0.40	2.172 ± 0.164		2.589 ± 0.146		2.127 ± 0.151
0.50	1.361 ± 0.169		1.409 ± 0.159		1.741 ± 0.161
0.60	0.647 ± 0.205		0.751 ± 0.085		1.117 ± 0.086

Table 24 : Normalised bacterial count (log CFU/g) of *S. typhi* in ground beef in the presence of various combinations of carvacrol (1.0 %), ascorbic acid (0.5 %) and tetrasodium pyrophosphate (0.1 %) at various irradiation doses for the determination of D_{10} value

Irradiation doses	Control	Carvacrol	Carvacrol + ascorbic acid	Carvacrol + tetrasodium pyrophosphate	Carvacrol + ascorbic acid + tetrasodium pyrophosphate
0.00	5.287 ± 0.187	5.286 ± 0.087	5.286 ± 0.056	5.286 ± 0.069	2.286 ±0.102
0.25		4.637 ± 0.271	4.751 ± 0.116	4.419 ± 0.167	5.143 ± 0.082
0.50	4.529 ± 0.267	3.537 ± 0.284	4.388 ± 0.101	3.831 ± 0.264	4.247 ± 0.130
0.60		2.792 ± 0.193	3.597 ± 0.074	2.031 ± 0.354	3.460 ± 0.064
0.75	4.211 ± 0.154	2.701 ± 0.173	3.141 ± 0.094	1.102 ± 0.155	2.923 ± 0.099
1.00	3.852 ± 0.188	0.841 ± 0.060	2.410 ± 0.061	0.474 ± 0.226	2.206 ± 0.040
1.50	2.514 ± 0.100				
2.00	1.597 ± 0.069				

Tableau 24a : Compte bactérien normalisé (log UFC/g) de *S. typhi* dans le bœuf haché en présence de différentes combinaisons de carvacrol (1.0 %), d'acide ascorbique (0.5 %) et tétrasodium pyrophosphate (0.1 %) à différentes doses d'irradiation pour la détermination de la D_{10}

Doses d'irradiation	Control	Carvacrol	Carvacrol + acide ascorbique	Carvacrol + tétrasodium pyrophosphate	Carvacrol + acide ascorbique + tétrasodium pyrophosphate
0.00	5.287 ± 0.187	5.286 ± 0.087	5.286 ± 0.056	5.286 ± 0.069	2.286 ±0.102
0.25		4.637 ± 0.271	4.751 ± 0.116	4.419 ± 0.167	5.143 ± 0.082
0.50	4.529 ± 0.267	3.537 ± 0.284	4.388 ± 0.101	3.831 ± 0.264	4.247 ± 0.130
0.60		2.792 ± 0.193	3.597 ± 0.074	2.031 ± 0.354	3.460 ± 0.064
0.75	4.211 ± 0.154	2.701 ± 0.173	3.141 ± 0.094	1.102 ± 0.155	2.923 ± 0.099
1.00	3.852 ± 0.188	0.841 ± 0.060	2.410 ± 0.061	0.474 ± 0.226	2.206 ± 0.040
1.50	2.514 ± 0.100				
2.00	1.597 ± 0.069				

Table 25 : Normalised bacterial count (log CFU/g) of *E. coli* in ground beef in the presence of carvacrol (1.0 %) in combination with tetrasodium pyrophosphate (0.1 %) under various atmospheres at various irradiation doses for the determination of D_{10} value

Irradiation doses	Air		CO_2		MAP[1]		Vacuum	
	Control	Carvacrol + tetrasodium pyrophosphate	Control	Carvacrol + tetrasodium pyrophosphate	Control	Carvacrol + tetrasodium pyrophosphate	Control	Carvacrol + tetrasodium pyrophosphate
0.00	5.372 ± 0.060	5.371 ± 0.065	5.370 ± 0.043	5.371 ± 0.067	5.371 ± 0.092	5.371 ± 0.064	5.371 ± 0.084	5.370 ± 0.052
0.10	4.775 ± 0.135	3.365 ± 0.145	4.913 ± 0.235	4.752 ± 0.092	3.217 ± 0.105	2.683 ± 0.164	4.857 ± 0.089	4.857 ± 0.100
0.15		2.331 ± 0.173	4.551 ± 0.060	4.574 ± 0.058	2.153 ± 0.137	1.266 ± 0.133		4.363 ± 0.132
0.20	3.840 ± 0.124	0.801 ± 0.085	4.352 ± 0.094	3.916 ± 0.275	3.075 ± 0.097	0.706 ± 0.235	3.971 ± 0.103	3.467 ± 0.054
0.25		0.463 ± 0.005		3.175 ± 0.104	2.683 ± 0.061	0.000 ± 0.000		3.182 ± 0.085
0.30	3.199 ± 0.126	0.000 ± 0.000	2.951 ± 0.125	2.474 ± 0.151	1.273 ± 0.209		3.199 ± 0.108	2.342 ± 0.091
0.40	2.246 ± 0.163		2.170 ± 0.135	2.009 ± 0.066	0.372 ± 0.089		1.861 ± 0.237	1.751 ± 0.085
0.50	1.403 ± 0.165		1.643 ± 0.081				1.283 ± 0.160	
0.60	0.725 ± 0.112						0.495 ± 0.097	

[1] 60% O_2–30% CO_2-10% N_2

Tableau 25a : Compte bactérien normalisé (log UFC/g) de *E. coli* dans le bœuf haché en présence de la combinaison de carvacrol (1.0 %) avec du tétrasodium pyrophosphate (0.1 %) sous différents atmosphères à différentes doses d'irradiation pour la détermination de la D_{10}

Doses d'irradiation	Air		CO_2		MAP[1]		Sous vide	
	Témoin	Carvacrol + tétrasodium pyrophosphate	Témoin	Carvacrol + tétrasodium pyrophosphate	Témoin l	Carvacrol + tétrasodium pyrophosphate	Témoin	Carvacrol + tétrasodium pyrophosphate
0.00	5.372 ± 0.060	5.371 ± 0.065	5.370 ± 0.043	5.371 ± 0.067	5.371 ± 0.092	5.371 ± 0.064	5.371 ± 0.084	5.370 ± 0.052
0.10	4.775 ± 0.135	3.365 ± 0.145	4.913 ± 0.235	4.752 ± 0.092	3.217 ± 0.105	2.683 ± 0.164	4.857 ± 0.089	4.857 ± 0.100
0.15		2.331 ± 0.173	4.551 ± 0.060	4.574 ± 0.058	2.153 ± 0.137	1.266 ± 0.133		4.363 ± 0.132
0.20	3.840 ± 0.124	0.801 ± 0.085	4.352 ± 0.094	3.916 ± 0.275	3.075 ± 0.097	0.706 ± 0.235	3.971 ± 0.103	3.467 ± 0.054
0.25		0.463 ± 0.005		3.175 ± 0.104	2.683 ± 0.061	0.000 ± 0.000		3.182 ± 0.085
0.30	3.199 ± 0.126	0.000 ± 0.000	2.951 ± 0.125	2.474 ± 0.151	1.273 ± 0.209		3.199 ± 0.108	2.342 ± 0.091
0.40	2.246 ± 0.163		2.170 ± 0.135	2.009 ± 0.066	0.372 ± 0.089		1.861 ± 0.237	1.751 ± 0.085
0.50	1.403 ± 0.165		1.643 ± 0.081				1.283 ± 0.160	
0.60	0.725 ± 0.112						0.495 ± 0.097	

[1] 60% O_2–30% CO_2-10% N_2

Table 26 : Normalised bacterial count (log CFU/g) of *S. typhi* in ground beef in the presence of carvacrol (1.0 %) in combination with tetrasodium pyrophosphate (0.1 %) under various atmospheres at various irradiation doses for the determination of D_{10} value

Irradiation doses	Air		CO_2		MAP[1]		Vacuum	
	Control	Carvacrol + tetrasodium pyrophosphate	Control	Carvacrol + tetrasodium pyrophosphate	Control	Carvacrol + tetrasodium pyrophosphate	Control	Carvacrol + tetrasodium pyrophosphate
0.00	5.287 ± 0.061	5.287 ± 0.072	5.284 ± 0.051	5.287 ± 0.070	5.287 ± 0.065	5.286 ± 0.000	5.288 ± 0.103	5.286 ± 0.092
0.10						4.339 ± 0.130		
0.15						3.200 ± 0.142		
0.20						2.696 ± 0.089		
0.25		4.419 ± 0.167		4.984 ± 0.155	3.939 ± 0.160	2.287 ± 0.063		4.822 ± 0.089
0.30						0.000 ± 0.000		
0.50	4.566 ± 0.166	3.831 ± 0.264	4.642 ± 0.111	4.010 ± 0.128	3.628 ± 0.051		4.819 ± 0.094	4.229 ± 0.088
0.75	4.238 ± 0.194	2.031 ± 0.354	4.500 ± 0.106	3.373 ± 0.134	2.597 ± 0.674		4.540 ± 0.070	2.837 ± 0.116
1.00	3.842 ± 0.089	1.102 ± 0.155	3.754 ± 0.088	2.745 ± 0.101	1.101 ± 0.664		3.430 ± 0.119	1.817 ± 0.194
1.25		0.474 ± 0.226		1.403 ± 0.175	1.606 ± 0.038			1.394 ± 0.092
1.50	2.504 ± 0.097		1.659 ± 0.103				1.765 ± 0.170	
2.00	1.574 ± 0.080		1.014 ± 0.068				1.019 ± 0.333	

[1] 60% O_2–30% CO_2-10% N_2

Tableau 26a : Compte bactérien normalisé (log UFC/g) de *S. typhi* dans le bœuf haché en présence de la combinaison de carvacrol (1.0 %) avec du tétrasodium pyrophosphate (0.1 %) sous différents atmosphères à différentes doses d'irradiation pour la détermination de la D_{10}

Doses d'irradiaiton	Air		CO_2		MAP[1]		Sous vide	
	Témoin	Carvacrol + tétrasodium pyrophosphate	Témoin	Carvacrol + tétrasodium pyrophosphate	Témoin	Carvacrol + tétrasodium pyrophosphate	Témoin	Carvacrol + tétrasodium pyrophosphate
0.00	5.287 ± 0.061	5.287 ± 0.072	5.284 ± 0.051	5.287 ± 0.070	5.287 ± 0.065	5.286 ± 0.000	5.288 ± 0.103	5.286 ± 0.092
0.10						4.339 ± 0.130		
0.15						3.200 ± 0.142		
0.20						2.696 ± 0.089		
0.25		4.419 ± 0.167		4.984 ± 0.155	3.939 ± 0.160	2.287 ± 0.063		4.822 ± 0.089
0.30						0.000 ± 0.000		
0.50	4.566 ± 0.166	3.831 ± 0.264	4.642 ± 0.111	4.010 ± 0.128	3.628 ± 0.051		4.819 ± 0.094	4.229 ± 0.088
0.75	4.238 ± 0.194	2.031 ± 0.354	4.500 ± 0.106	3.373 ± 0.134	2.597 ± 0.674		4.540 ± 0.070	2.837 ± 0.116
1.00	3.842 ± 0.089	1.102 ± 0.155	3.754 ± 0.088	2.745 ± 0.101	1.101 ± 0.664		3.430 ± 0.119	1.817 ± 0.194
1.25		0.474 ± 0.226		1.403 ± 0.175	1.606 ± 0.038			1.394 ± 0.092
1.50	2.504 ± 0.097		1.659 ± 0.103				1.765 ± 0.170	
2.00	1.574 ± 0.080		1.014 ± 0.068				1.019 ± 0.333	

[1] 60% O_2–30% CO_2-10% N_2

Table 27 : Normalised bacterial count (log CFU/g) of *E. coli* in ground beef in the presence of carvacrol (1.0 %) in combination with tetrasodium pyrophosphate (0.1 %) under two irradiation temperatures (4°C and –80°C) at various irradiation doses for the determination of D_{10} value

Irradiation doses	4°C		- 80°C	
	Control	Carvacrol + tetrasodium pyrophosphate	Control	Carvacrol + tetrasodium pyrophosphate
0.00	5.372 ± 0.060	5.371 ± 0.065	5.370 ± 0.046	5.370 ± 0.043
0.10	4.775 ± 0.135	3.365 ± 0.145	4.965 ± 0.110	4.736 ± 0.084
0.15		2.331 ± 0.173		
0.20	3.840 ± 0.124	0.801 ± 0.085	4.373 ± 0.093	4.181 ± 0.082
0.25		0.463 ± 0.005		
0.30	3.199 ± 0.126	0.000 ± 0.000	3.926 ± 0.096	3.363 ± 0.091
0.40	2.246 ± 0.163		3.188 ± 0.099	2.125 ± 0.087
0.50	1.403 ± 0.165			1.496 ± 0.242
0.60	0.725 ± 0.112		2.728 ± 0.110	0.947 ±0.229
0.70			2.366 ± 0.095	

Tableau 27a : Compte bactérien normalisé (log UFC/g) de *E. coli* dans le bœuf haché en présence de la combinaison de carvacrol (1.0 %) avec du tétrasodium pyrophosphate (0.1 %) à deux températures (4°C and –80°C) à différentes doses d'irradiation pour la détermination de la D_{10}

Doses d'irradiation	4°C		- 80°C	
	Témoin	Carvacrol + tetrasodium pyrophosphate	Témoin	Carvacrol + tétrasodium pyrophosphate
0.00	5.372 ± 0.060	5.371 ± 0.065	5.370 ± 0.046	5.370 ± 0.043
0.10	4.775 ± 0.135	3.365 ± 0.145	4.965 ± 0.110	4.736 ± 0.084
0.15		2.331 ± 0.173		
0.20	3.840 ± 0.124	0.801 ± 0.085	4.373 ± 0.093	4.181 ± 0.082
0.25		0.463 ± 0.005		
0.30	3.199 ± 0.126	0.000 ± 0.000	3.926 ± 0.096	3.363 ± 0.091
0.40	2.246 ± 0.163		3.188 ± 0.099	2.125 ± 0.087
0.50	1.403 ± 0.165			1.496 ± 0.242
0.60	0.725 ± 0.112		2.728 ± 0.110	0.947 ±0.229
0.70			2.366 ± 0.095	

Table 28 : Normalised bacterial count (log CFU/g) of *S. typhi* in ground beef in the presence of carvacrol (1.0 %) in combination with tetrasodium pyrophosphate (0.1 %) under two irradiation temperatures (4°C and –80°C) at various irradiation doses for the determination of D_{10} value

Irradiation doses	4°C		- 80°C	
	Control	Carvacrol + tetrasodium pyrophosphate	Control	Carvacrol + tetrasodium pyrophosphate
0.00	5.287 ± 0.061	5.287 ± 0.072	5.287 ± 0.077	5.287 ± 0.078
0.25		4.419 ± 0.167		
0.50	4.566 ± 0.166		5.007 ± 0.055	3.684 ± 0.113
0.75	4.238 ± 0.194	3.831 ± 0.264		2.476 ± 0.182
1.00	3.842 ± 0.089	2.031 ± 0.354	4.219 ± 0.082	1.821 ± 0.171
1.25		1.102 ± 0.155		0.886 ± 0.277
1.50	2.504 ± 0.097	0.474 ± 0.226	3.639 ± 0.082	0.414 ± 0.175
2.00	1.574 ± 0.080		2.551 ± 0.101	
2.50			2.016 ± 0.080	
3.00			1.165 ± 0.087	

Tableau 28a : Compte bactérien normalisé (log UFC/g) de *S. typhi* dans le bœuf haché en présence de la combinaison de carvacrol (1.0 %) avec du tétrasodium pyrophosphate (0.1 %) à deux températures (4°C and –80°C) à différentes doses d'irradiation pour la détermination de la D_{10}

Doses d'irradiation	4°C		- 80°C	
	Témoin	Carvacrol + tétrasodium pyrophosphate	Témoin	Carvacrol + tétrasodium pyrophosphate
0.00	5.287 ± 0.061	5.287 ± 0.072	5.287 ± 0.077	5.287 ± 0.078
0.25		4.419 ± 0.167		
0.50	4.566 ± 0.166		5.007 ± 0.055	3.684 ± 0.113
0.75	4.238 ± 0.194	3.831 ± 0.264		2.476 ± 0.182
1.00	3.842 ± 0.089	2.031 ± 0.354	4.219 ± 0.082	1.821 ± 0.171
1.25		1.102 ± 0.155		0.886 ± 0.277
1.50	2.504 ± 0.097	0.474 ± 0.226	3.639 ± 0.082	0.414 ± 0.175
2.00	1.574 ± 0.080		2.551 ± 0.101	
2.50			2.016 ± 0.080	
3.00			1.165 ± 0.087	