Patogenfrit kød

Perspektiveret redegørelse

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2. Forord

Denne redegørelse har til formål at kortlægge dekontamineringsløsninger, der er relevante for kødindustrien, med henblik på at fremstille patogenfrit kød. Fokus har været på teknologier, hvor der har været påvist effekt på fersk kød overfor *Listeria* og *Salmonella* eller hvor der formodes at være reelt potentiale herfor. For hver teknologi er der en beskrivelse af den mikrobielle virkemåde, hvordan interventionen kan påvirke ikke-mikrobielle kvalitetsparametre og eventuelle erfaringer fra industrielle applikationer. Hvis der er særligt interessante resultater fra litteraturen, er disse kort beskrevet. I det omfang der foreligger oplysninger, medtages information om kommercielle udbydere af teknologien, vurdering af krav til effekt samt lovgivningsmæssige perspektiver. Til sidst gives et overordnet overblik over hvordan teknologien vurderes at påvirke bæredygtighed og miljø efterfulgt af en opsummering af fordele og ulemper ved den specifikke løsning.

3. Projektets baggrund

De mikrobiologiske krav til fersk kød øges konstant. Det betyder, at forespørgsler på fersk kød fri for forskellige patogene bakterier stiger. "Fri for *Salmonella*, *Campylobacter* og *VT E. coli*" er kendte produktkrav, og kravet om "*Listeria*-frit" er i stigning. Det vurderes derfor, at patogenfrit kød vil være "licence to sell" indenfor en horisont på 10 år. Dette krav er, med de nuværende slagte-, udbenings- og opskæringsprocesser, ikke muligt at opfylde. Der er derfor behov for at se ind i, hvordan det i fremtiden kan gøres muligt at producere patogenfrit kød på danske slagterier. Det skal bl.a. vurderes, hvor i produktionskæden det vil være mest hensigtsmæssigt at sætte ind, samt hvilke tiltag der vil være bedst egnede i en produktion, hvor også bæredygtighed er en vigtig parameter

4. Projektets formål og mål (iflg. projektansøgning)

Formålet er et identificere, hvordan produktion af patogenfrit kød kan implementeres. Målet nås ved perspektivering af muligheder for produktion af patogenfrit kød.

5. Aktiviteter

Projektets aktiviteter skal understøtte udarbejdelse af en perspektiveret redegørelse for, hvordan det kan gøres muligt at producere patogenfrit kød indenfor de næste 10 år. Projektaktiviteterne skal afdække, om der mangler viden og teknologi for at nå målet om at producere patogenfrit kød. Aktiviteterne skal koordineres tæt med repræsentanter fra slagterierne for løbende at vurdere, hvad der vil give værdi, og hvad der vil kunne lykkes i produktionen; herunder en prioritering af, hvilke løsningsmuligheder der vil kunne lykkes indenfor en kort, mellemlang og langsigtet tidshorisont. Projektet vil blive indledt med en workshop/brainstorm, hvor løsningsrummet identificeres, og hvor der fx diskuteres krav til teknologiløsninger, fordele og ulemper ved markedsføring og salg af patogenfrit kød samt nedenstående eksempler på emner:

- *Hvor i værdikæden vil tiltag være perspektivrige, og hvor vil en indsats være uden effekt?*
  - Findes der data og/eller erfaringer fra forsøg med andre slagteteknikker fx afslagtning af hoveder, og kan det give inspiration til nytænkning? Kan gode såvel som dårlige erfaringer eller forsøgsresultater give anledning til nytænkning? Er det muligt at ændre på slagtemetode både i forhold til nuværende udstyr og kundernes krav?
  - Er der udenlandske erfaringer, der kan bygges videre på (fx Holland)?
  - Er der erfaringer fra andre industrier, som danske slagterier kan trække på (fx fjerkræ)?
  - I forhold til at drage erfaringer fra andre områder (udland, industrier), hvori ligger de specifikke udfordringer for (dansk) gris?

- *Hvor effektive skal nye processer være i forhold til eliminering af patogener eller forebyggelse af krydskontaminering?*
  - Er der nogen specifikke mål for, hvad 'patogen-frit' betyder i andre lande/industrier? På baggrund af detektionsgrænsen i de traditionelle mikrobiologiske metoder er ikke påvist i 25 g ofte = fravær af mikroorganismen. Men hvad må risikoen for en positiv prøve være?
  - Hvilke krav stiller det til effekten af en dekontamineringsteknologi set i forhold til det nuværende niveau af patogener? Er 2 log reduktion nok, eller kræves der 5 log reduktion, for at sandsynligheden for at påvise patogener er lav nok? Skal dekontamineringen bruges som en ekstra sikkerhed ved procesfejl ved slagtningen, og skal der anvendes en eller flere teknologier?
  - Er der publikationer på området, som beskriver frekvensen af krydskontaminering, basisniveauer i forskellige processer og måske effekten af forskellige tiltag?

- *Bliver dekontamineringsteknologiers antimikrobielle effekt, CSR-aftryk (miljø og bæredygtighed) samt omkostningerne til ændrede processer og investering i ny teknologi en show-stopper?*
  - Vurdering af forskellige dekontamineringsmetoder, som bruges i dag i andre lande (fx Australien, NZ og USA) eller andre industrier (fx fjerkræ, lam, ko)
  - Vurdering af up-coming teknologier fra litteraturen (fx bakteriofager, UV, m.m.)
  - Vurdering af effekten af dekontaminering vs. rekontaminering via operatører, dvs. i hvor høj grad spiller træning af medarbejdere og Food Safety Culture en rolle?
  - Diskussion af omkostninger vs. den forventede merindtægt (for patogenfrit kød) – kan der beregnes et breaking-point, hvor merindtægten dækker meromkostningerne?
  - Diskussion af mulighederne for, at der gives mulighed for dekontaminering af produkter (fra myndighedernes side).
  - Alle vurderinger indarbejdes i skema med kolonner for: metodebeskrivelse (kort), anvendelsesområde, forventet effekt (reduktion af patogener), forventet effekt (CSR-aftryk), forventede omkostninger (investering), forventede omkostninger (daglig drift).

- *Hvor i processen vil en dekontaminering være mest hensigtsmæssig – fx produkt eller udstyr, slagtegang eller opskæring? En anden mulighed er at bruge dekontaminering til renholdelse under produktion af fersk kød som ved produktion af forarbejdede kødprodukter ved fx slicening af pålæg.*

- Vurdering af, om et RTE-approach for fersk kød er realistisk og effektivt nok til at nå niveauet for patogenfrit.
- Vurdering af, om alt fra en slagtegang (opskæring) skal være patogenfrit, eller om der er større perspektiver i at udvælge særlige udskæringer til at være patogenfrie, og om det rent praktisk kan lade sig gøre.

Den perspektiverede redegørelse udarbejdes på baggrund af indsamling af viden fra bl.a. litteratur, teknologileverandører, slagterier i andre lande samt erfaringer fra produktion af kød fra andre dyrearter og fra produktion af forædlede produkter. Hvis dekontaminering identificeres som en relevant løsning, igangsættes test af de mest perspektivrige dekontamineringsmetoder under hensyntagen til kundekrav, lovgivning og den grønne omstilling. Hvis de indledende test giver lovende resultater, kan et nyt projekt igangsættes med opskalering og implementering af teknologien.

6. Kravspecifikation

Efter grundig litteratursøgning og videnindsamling på konferencer mv. blev der i Q3 2022, i tæt samarbejde med dialoggruppen, via en dybdegående SWOT-analyse, genereret en velafgrænset kravspecifikation for resten af projektforløbet.

Hvor udgangspunktet for projektet var relativt bredt formuleret, var det nødvendigt for at sikre et brugbart resultat af projektet at få konkretiseret mål, formål og succeskriterier. Kravspecifikationen præciserer hvad der fx menes med 'patogen' i denne sammenhæng og fokuserer indsatsen omkring et produkt som der er konkret viden om at der vil være afsætning for. Den præciserer desuden hvad dialoggruppens kriterier for succes er, med en graduering af hvilket resultat der vil betragtes som 'rigtig godt', 'forventet' og 'acceptabelt'.

6.1. Mål – hvad skal vi opnå?

At være i stand til at producere brystflæsk, der er 'fri for' *Listeria* og *Salmonella,* når det forlader virksomheden. 'Fri for' defineres som ikke detekteret i 25 g.

6.2. Formål – hvorfor gør vi det?

Formålet er dels at undersøge om der findes en metode til at dekontaminere til det definerede 'fri for' niveau og dels hvad implementeringen af en sådan metode vil have af økonomiske fordele, ulemper og konsekvenser.

6.3. Kommentarer og overvejelser vedrørende kravspecifikationen

Det er altså besluttet at fokusere indsatsen på at finde en måde hvorpå man kan garantere, at der ikke vil kunne detekteres *Listeria* og/eller *Salmonella* i en 25 grams prøve udtaget fra 'patogenfrit' brystflæsk. Der blev valgt at fokusere på brystflæsk ud fra en konkret viden om en bacon-producent i Holland, der efterspørger en 'patogenfri' råvare.

De teknologier der vil blive gennemgået i afsnit 7, vil alle sigte mod en afsluttende dekontaminering, der sikrer at produktet når det forlader virksomheden (slagteriet eller dekontamineringsfaciliteten) har fået inaktiveret evt. forekommende celler af *Listeria* og/eller *Salmonella* til et ikke-detekterbart niveau.

Det er vigtigt for at opnå både myndighedernes og B2B kundernes godkendelse, samt forbrugernes accept, at det kan dokumenteres at dekontamineringen ikke sker for at dække over en uhensigtsmæssig behandling af produktet tidligere i processen. Det skal altså kunne dokumenteres, at der både på landsplan og i den individuelle virksomhed gøres en stor indsats for at undgå forurening med *Listeria* og *Salmonella* i produktionsmiljøet (fx via hyppige evalueringer med Listeria Action Card'et), at rengøringsprocedurerne er effektive og tilstrækkelige (fx ved rutinemæssig verifikation af effekten af den implementerede rengøring) og at de indgående råvarer generelt har en lav forekomst af *Listeria* og *Salmonella* (fx via salmonellaovervågningsdata og in-process rutineprøver for *Listeria* på produktniveau).

7. Teknologier i alfabetisk orden

Anvendte forkortelser

LAB	Lactic acid bacteria	LINAC	Linear accelerator
GRAS	Generally recognized as safe	DC	Direct current
FDA	Food and Drug Administration (i USA)	CW	Cockcroft–Walton
EFSA	European Food Safety Authorities	FDA	Food and Drug Administration (in USA)
GRAS	Generally recognized as safe	USDA	United States Department of Agriculture
RTE	Ready-to-eat	FSIS	Food Safety Inspection Services (under USDA)
HPP	High pressure processing	PAA	Peracetic acid
UV	Ultraviolet	PEF	Pulsed electric field
HPP	High pressure processing	WHC	Water holding capacity
UHP	Ultra high pressure (processing)	SSO	Specific spoilage organism
HHP	High hydrostatic pressure (processing)	NTAP	Nonthermal atmospheric plasma
eBeam	Electron beam	CP	Cold plasma

7.1. Bakteriofager

Dokumenteret effekt på fersk kød	*Listeria* reduktion 1-2,3 log	*Salmonella* reduktion 1-4 log	Holdbarheds-forlængelse 4 dage[1]

Kødtyper testet	Gris	Okse	Fjerkræ	Andet
	X	X	X	RTE, fisk

(Se detaljer i tabellerne i bilag 1)

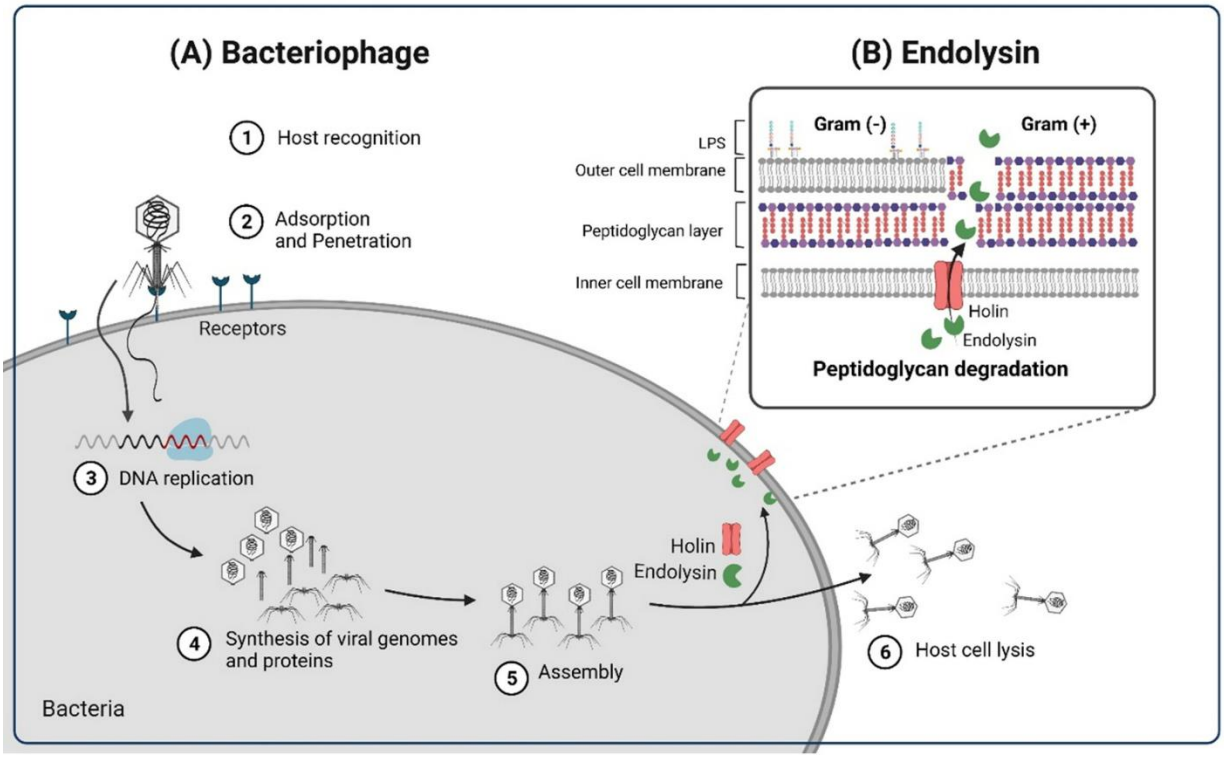
Virkemåde

Bakteriofager er de mest talrige og udbredte biologiske enheder i biosfæren, herunder fødevarer og miljø, med en anslået population på 10^{31}. Bakteriofagers virkemåde er baseret på en 'seek-and-destroy' taktik og er grundlæggende en virus for bakterier. Når en bakteriofag binder sig til en bakteriecelle (vært), trænger den gennem cellevæggen og injicerer sit eget DNA i værtscellen, hvilket effektivt overtager værtscellen og ødelægger dens evne til at fungere eller replicere (se figur 3A). Bakteriofager er meget specifikke og bruges derfor ofte i 'cocktails' designet til den specifikke anvendelse og de organismer der sigtes mod. Bakteriofager er ustabile og mister hurtigt deres styrke.

[1] *Brochothrix*-specifik bakteriofag anvendt på fedtvæv fra gris. Holdbarhedsforlængelse fra 4 til 8 dage (Kazi & Annapure 2016)

Bakteriofagkodede enzymer (fx endolysin) er lige så specifikke som den bakteriofag, de genereres af, og har en virkningsmåde svarende til bakteriociner, da de forstyrrer cellevæggen og/eller membranen i deres målorganisme (se figur 3B). Bakteriofagkodede enzymer er relativt stabile.

Bakteriofager og endolysiner kan med fordel anvendes i kombination med andre ikke-termiske processer, fx UV-C lys, HPP og/eller bakteriociner.



Figur 3. Virkemåde for bakteriofag (A) hhv. endolysin (B) (Lee et al 2022).

Indvirkning på ikke-mikrobielle kvalitetsparametre

Bakteriofager og bakteriofagkodede enzymer forventes ikke at forårsage skadelige eller uhensigtsmæssige virkninger på ikke-mikrobielle kvalitetsparametre. Der kan forventes en statisk eller positiv effekt på den sensoriske evaluering, der kan tilskrives den antimikrobielle effekt og deraf følgende undertrykkelse af evt. ubehagelig smag/lugt, der metaboliseres fra fordærverorganismer.

Industrielt brug

Flere kommercielle produkter er markedsført i USA (som fødevaretilsætningsstoffer) og andre lande (typisk som tekniske hjælpestoffer), fx produkter mod *Listeria monocytogenes* (ListShield™ og PhageGuard Listex), *Salmonella* (SalmoFresh™ og PhageGuard S) og *Escherichia coli* (EcoShield™). Flere af produkterne kan også bruges til dekontaminering af overflader.

Kommercielle udbydere

- Micreos Food Safety (Holland) (https://phageguard.com/).

Resultater af særlig interesse

- Kød
    - En kombination af tre hurdles (bakteriofag, HPP og en bakteriocinproducerende stamme) anvendt i en fermenteret pølse model, opnåede en 5-log reduktion (Komora et al. 2021)
    - En reduktion på ca. 2,3 log blev vist i bakteriofag-behandlet oksekød i løbet af en 15-dages opbevaringsperiode (Ishaq et al. 2020)
    - En 2-log reduktion af *Salmonella Derby* og *Listeria monocytogenes* på fersk grisekød efter behandling med en 1% fagsuspension (PhageGuard S hhv. PhageGuard Listex). *S. Typhimurium* var ikke følsom overfor fagsuspensionen (Jacobsen 2019)
- Fisk
    - Nedsænkning af regnbueørred i 30 sek. i en opløsning af en bakteriofagcocktail (≥8 log PFU/ml), forsinkede bakterielt fordærv med 3 dage (fra 10,5 dage til 13,5 dage), sammenlignet med kontrollen (Zulkarneev et al. 2019)

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området.
- EU: Anvendelsen af bakteriofager i animalske fødevarer blev evalueret af Det Europæiske Fødevaresikkerhedsagentur (EFSA) i 2009 og brugen af PhageGuard Listex til fjernelse af *L. monocytogenes* fra rå fisk blev vurderet i 2012. I begge undersøgelser konkluderede EFSA, at bakteriofager er harmløse for forbrugerne, men at det er ikke klart, om de kan beskytte mod rekontaminering af fødevarer.
- USA: ListShield (Intralytix, Inc., Baltimore, MD, USA) var det første bakteriofagbaserede produkt der opnåede godkendelse af FDA og USDA som et fødevaretilsætningsstof til kontrol af *L. monocytogenes* i spiseklare fødevarer (2006) og desuden tildelt GRAS-status af FDA i 2014. Siden da er andre bakterofagbaserede produkter som EcoShield (Intralytix), SalmoFresh (Intralytix) og Agriphage (OmniLytics Inc., Sandy, UT, USA) også blevet godkendt til kommerciel brug i fødevaresikkerhed.
- Øvrige lande: Bakteriofager er godkendt som tekniske hjælpestoffer i fødevareforarbejdning og -håndtering i flere lande, inkl. Canada (iLONO), Australien, New Zealand, Israel (Ref: 70275202) og Schweiz.

Indvirkning på bæredygtighed og miljø

- Energi LAV
- Vand LAV
- Kemi INGEN
- Arbejdsmiljø INGEN

Krav til effekt

- Tid: >0,5 log indenfor 1-2 minutter, max. effekt (1-2 log) efter 6-18 timer (PhageGuard S)
- Afstand: kontakt
- Dosis: 10^6 – 10^{10} PFU/ml, 1-2 ml/250 cm^2 eller 1-4 ml/450 g (ListShield)

Omkostninger

- Anskaffelse: kommerciel sprayer
- Drift: forhandlet pris på fager (brugsopløsning ca. 1%), energiforbrug sprayer

Fordele

- Lille kapitalinvestering, Kosher/Halal, OMRI (økologisk), naturligt, non-GMO, effektivt i et bredt temperaturinterval (2-42°C), ændrer ikke fødevarens smag, aroma eller næringsværdi

Ulemper

- Høj mutationsrate (bakteriofager), skal fortyndes inden brug, skal opbevares køligt (2-8°C), begrænset effekt ved brug alene

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7.2. Bestråling

De mest almindeligt anvendte bestrålingsteknologier er gamma, X-ray (røntgen) og elektronstråler (eBeam) stråling. Alle tre teknologier er kategoriseret som ioniserende strålingsteknologier, hvilket betyder, at de kan 'sparke' elektroner ud af deres orbitalskaller omkring atomer og derved 'ionisere' atomerne. Hvor gammabestråling er afhængig af radioaktive isotoper (f.eks. cæsium-137 eller kobolt-60), genereres røntgen- og eBeam-bestråling fra kommerciel elektricitet gennem 'lineære acceleratorer' (LINAC).

Teknologierne

Elektronstråler

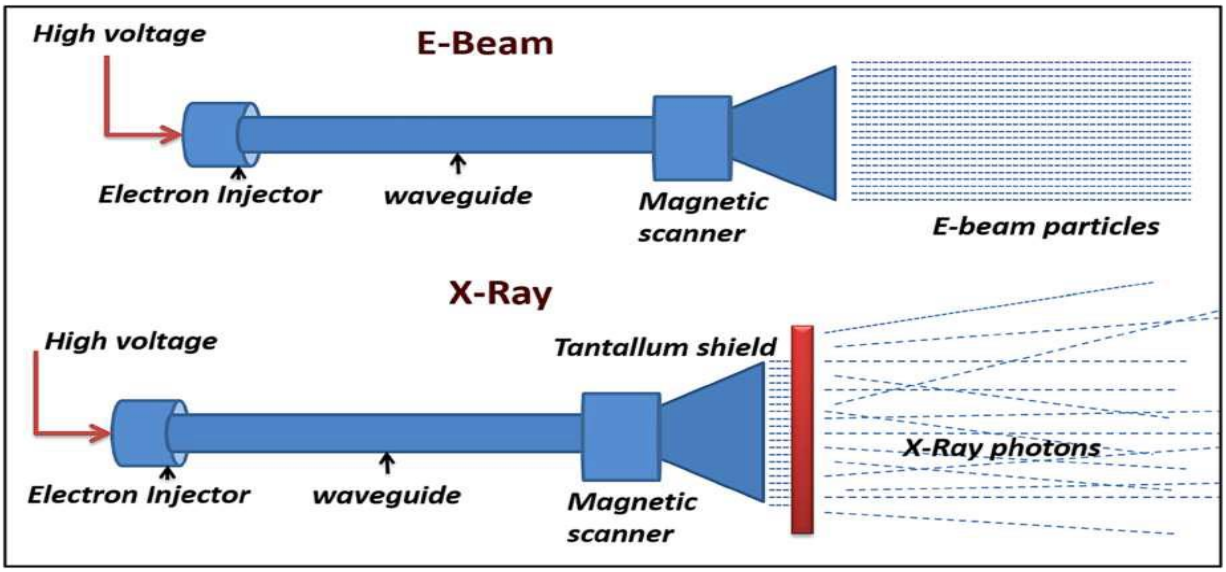
Elektronstråler (eBeams) genereres med et udstyr (en accelerator), der omdanner kommerciel elektricitet til en højenergistrøm af eBeam-partikler (se figur 4). Der er tre kommercielt tilgængelige typer eBeam-acceleratorer: DC (jævnstrøm), CW (Cockcroft-Walton) og pulserende acceleratorer, som hver har forskellige fordele og ulemper (se tabel 1).

Gammastråler

Radioaktive kildematerialer såsom cæsium-137 og kobolt-60 (produceret i atomreaktorer) er de vigtigste kilder til gammabestråling. Gammabestråling består hovedsageligt af fotoner, som ikke har nogen masse. Dette gør det muligt for gammabestrålingen let at trænge gennem materialer med varierende densitet, hvilket overgår hvad røntgen og eBeam stråler kan præstere. Dosishastigheden for gammabestråling, dvs. den hastighed, hvormed energien afsættes i målmaterialet, er imidlertid betydeligt lavere end røntgen- eller eBeam-processer i kommerciel skala.

Røntgenstråler

Røntgenstråler fremstilles af LINAC- eller Rhodotron-stil acceleratorer. Røntgengenerering fra en elektronstråle i en LINAC er baseret på placeringen af et materiale med meget høj atommasse (fx tantal eller guld) direkte i strømbanen af en højenergi elektronstråle (se figur 4). Kollisionen af højenergielektroner resulterer i dannelsen af røntgenfotoner. Røntgenfotoners evne til at trænge gennem materialer svarer næsten til gammabestrålingens (dvs. bedre end eBeam), men både fotonenergi og dosishastighed for røntgenstråler er meget højere end for kobolt-60-baseret gammabestråling.



Figur 4. Skematisk repræsentation af principperne bag eBeam- og røntgenstråling ved brug af en accelerator af LINAC-typen (Pillai & Shayanfar, 2017)

Tabel 1. Sammenligning af kommercielt anvendte DC-, CW- og pulserende acceleratorer (Pillai & Shayanfar, 2017)

Parameter	DC-accelerator	CW-accelerator	Pulserende accelerator
Type	Dynamitron	Rhodotron	LINAC
Maks. anvendt energi	5 MeV	7,5 – 10 MeV	10 MeV
Effekt[1)]	Høj (op til 100 kW)	Høj (op til 800 kW)	Begrænset (op til 20 kW)
Effektivitet i brug af el	Høj	Middel	Lav
Fysisk størrelse	Stor	Middel	Lille

[1)] Oversættes til mulige linjehastigheder (høj effekt = høj linjehastighed)

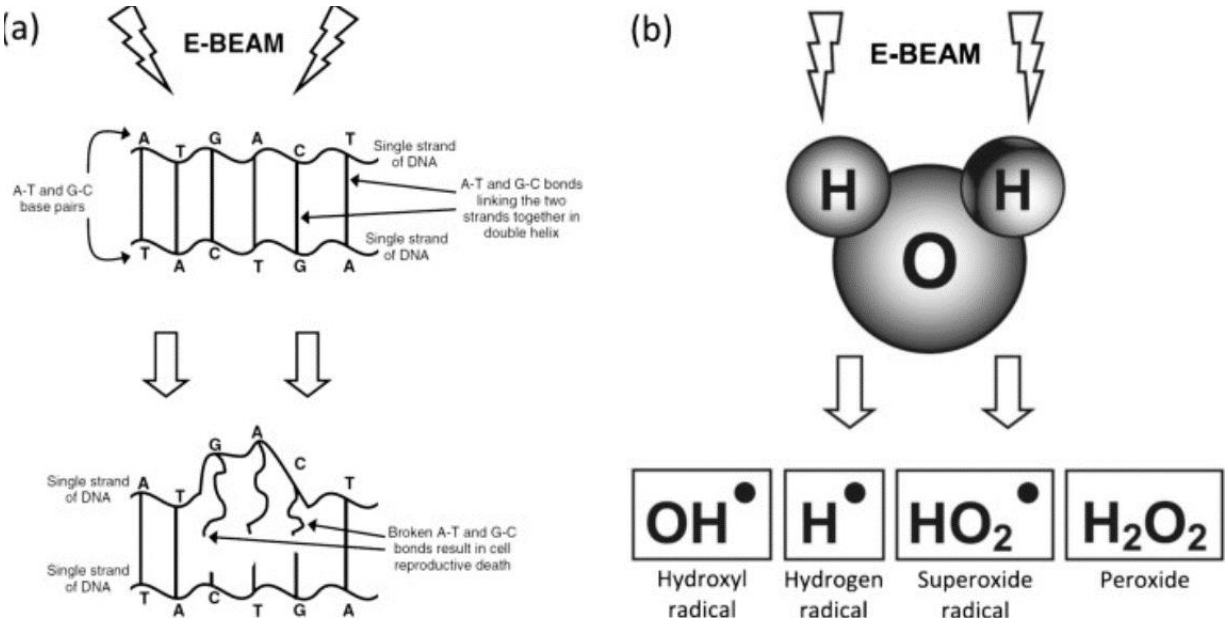
Tabel 2. Opsummering af gennemtrængningsdybde og effektivitet af de tre ioniserende strålingsteknologier brugt til behandling af fødevarer (Aymerich et al. 2008).

	Gamma ray	X-ray	E-beam
Power source (kW)	~50	25	35
Source energy (MeV)	1.33	5	5–10
Processing speed[a] (tonnes/h)	12	10	5–10
Penetration depth (cm)	80–100	80–100	8–10
Dose uniformity ratio	~1.7	~1.5	Moderate
Dose rate (kGy/h)	Low	High	High

[a] Processing speed to deliver a dose rate of 4 kGy.

Virkemåde

Bestråling forårsager direkte og indirekte DNA-beskadigelse af de mikrobielle celler. Direkte skade opstår, når fotoner eller elektroner direkte støder på DNA-molekylet og forårsager enkelt- og dobbeltstrengsbrud, når elektroner fjernes. Indirekte DNA-skader sker, når elektron, der udstødes fra deres orbitalskal, rammer elektroner i tilstødende atomer, hvilket skaber en række tilsvarende ioniseringshændelser. Når ioniserende stråling møder vandmolekyler, ioniseres vandmolekylet med det resultat, at hydrolyse opstår, og der dannes stærkt reaktive (men ekstremt kortvarige) frie radikaler. De frie radikaler forårsager yderligere skade på DNA-molekylerne (se figur 5).



Figur 5. (a) direkte virkning (DNA/RNA skader); (b) indirekte virkning (frie radikaler angriber cellemembranen) (Lung et al. 2015)

Effekten kan variere noget alt efter målorganisme, matrix og temperatur. Ahn et al. (2013) har samlet D-værdierne (i kGy) for forskellige patogener, kødprodukter og temperaturer (tabel 3). Det er værd at notere, at organismer der er særligt miljøtilpassede samt endosporer af *Clostridium* og *Bacillus* udviser en høj resistens overfor bestråling (se fx D-værdien for sporer af *Clostridium sporogenes* i tabel 3).

Tabel 3. D-værdier for fødevarebårne patogener (Ahn et el. 2013)

Pathogen	D10 (kGy)	Medium	Temperature (°C)
Listeria monocytogenes	0.42 to 0.44	Ground pork	0 to 5
Clostridium perfringens	0.826	Ground pork	10
Salmonella spp.	0.61 to 0.66	Ground beef	4
Escherichia coli O157H7	0.24	Beef	2 to 4
Campylobacter jejuni	0.11 to 0.19	Ground turkey	0 to 4
Staphylococcus aureus	0.40 to 0.66	Chicken	0
Vibrio parahemoliticus	0.053 to 0.357	Crab meat	24
Yersina enterocolitica	0.164 to 0.204	Ground pork	10
Clostridium sporogenes (spore)	6.3	Beef fat	4
Moraxella phenylpyruvica	0.63 to 0.88	Chicken	4
Pseudomonas putida	0.08 to 0.11	Chicken	4
Streptococcus fecalis	0.65 to 0.7	Chicken	4

Indvirkning på ikke-mikrobielle kvalitetsparametre

Strukturelle proteiner, katalytiske proteiner (enzymer) og de fleste vitaminer beskadiges ikke ved de doser, der almindeligvis anvendes til bestråling af fødevarer. Eksponering for meget høje doser kan dog forårsage skade på makromolekyler såsom proteiner.

Industrielt brug i EU

I tabel 4 er listet de fødevarer, der blev kommercielt bestrålet i EU i 2011. De fødevarer, der oftest dekontamineres med bestråling er krydderier og urter, men i visse lande (Belgien, Frankrig og Nederlandene) anvendes bestråling til et bredere udvalg af fødevarer.

Tabel 4. Fødevarer der bliver bestrålet kommercielt i forskellige EU-lande (data fra 2011) (Pillai & Shayanfar, 2017).

EU country	Food and food ingredients irradiated
Belgium	Dehydrated blood, egg white, fish, shellfish, shrimp, frog legs, gum Arabic, herbs and spices, poultry, rice meal, vegetables
Czech Republic	Herbs and spices
Germany	Herbs and spices
Estonia	Herbs and spices
Spain	Herbs and spices
France	Frog legs, gum Arabic, herbs and spices, poultry
Hungary	Herbs and spices
The Netherlands	Egg whites, fish, shellfish, shrimp, frog legs, herbs and spices, poultry, dehydrated products
Poland	Herbs and spices

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området.
- EU: Levnedsmiddelbestråling er lovlig (og endda anbefalet) i EU gennem direktiv 1999/2/EF og direktiv 1999/3/EF, hvor fødevarer og fødevareingredienser, der må behandles med bestråling, er listet. Syv medlemsstater har en udvidet liste over fødevarer og fødevareingredienser, der må bestråles. I 2021 var der 24 godkendte bestrålingsanlæg i 14 EU-lande. De mest bestrålede produkter er frølår, fjerkræ og tørrede aromatiske urter, krydderier og grøntsager krydderier. De forordninger, der blev gennemført i 2011, opnåede ikke en harmonisering af lovgivningen om bestråling i hele EU idet nationale agenturer stadig kunne udstede tilladelser og forbud mod andre bestrålede fødevarer end urter og krydderier. Belgien, Tjekkiet, Frankrig, Italien, Nederlandene og Polen har en national liste over tilladelser til behandling af levnedsmidler med ioniserende stråling. Mærkning er påkrævet.
- USA: I USA betragtes bestråling af fødevarer som et tilsætningsstof og er reguleret af 1958 Food Additives Amendment of the Food, Drug and Cosmetics Act. Fødevarer, der må behandles med bestråling, omfatter frisk frugt/grøntsager, kød (fra ko, svin, fjerkræ, får, ged), bløde skaldyr, hele

æg, krydderier, frø, mel og forskellige emballagematerialer. Anvendelsen er begrænset af specifikke formål med bestråling og maksimale doser. Mærkning påkrævet (radura logo).

- Kina: Blandt alle lande bestråler Kina den største mængde fødevarer. Sandsynligvis mere end 250.000 tons kyllingefødder, der sælges som snacks, bestråles årligt. Tilladte fødevarer og emballagematerialer, maksimale doser og mærkningskrav er reguleret af nationale standarder.
- Indien: Reglerne om bestråling af fødevarer i Indien blev revideret i 2016. Forordningen indeholder en liste over tilladte levnedsmidler og emballagematerialer samt bestrålingsformål og minimums-/maksimumsdoser. Som i USA skal bestrålede fødevarer mærkes med radura-logoet.

7.2.1. eBeam

Dokumenteret effekt på fersk kød	*Listeria* reduktion ? log	*Salmonella* reduktion 2-5 log	Holdbarheds-forlængelse 14 dage[2]

Kødtyper testet	Gris	Okse	Fjerkræ	Andet
		X	X	RTE, fisk, skaldyr

(Se detaljer i tabellerne i bilag 2)

Industrielt brug

eBeam teknologien bruges i USA til bestråling af hakket oksekød (fås hos butikskæderne Schwan's og Wegmans)

eBeam kan med fordel bruges i forbindelse med aseptisk pakning og der findes kommercielt tilgængeligt udstyr specielt til dette (figur 6).

Figur 6. Lavenergi eBeam kilde til brug ved lavenergi overfladesterilisation (Pillai & Bhatia, 2018).

[2] García-Márquez et al. 2012

Kommercielle udbydere

eBeam tunnel (hyldevare) kommercielt tilgængelig hos SKAN (SKAN_datablad_tunnel, 2022). Tunnelen er udviklet til pharma-industrien til sterilisering af emballage (aseptisk pakning).

eBeam udstyr (specialfremstillet) kan rekvireres hos kommercielle udbydere, fx Wasik Associates, Inc. (www.wasik.com) eller Global Beam Technologies AG (www.sst-ebeam.com). Andre udstyrsproducenter: ScanTech Sciences Inc., Atlanta; GrayStar Inc., Mt. Arlington, N.J., IBA Industrial Inc., Edgewood, N.Y., Russia-based Tecleour; Ebeam Technologies, Switzerland; Mevex, Canada; Iotron Industries, Canada.

Testfacilitet findes fx hos STFC Daresbury Laboratories (https://www.ukri.org/about-us/stfc/locations/daresbury-laboratory/) eller STERIS Applied Sterilization Technologies (https://www.steris-ast.com/solutions/electron-beam-irradiation/).

Tabel 5. Klassificering af anvendelser af eBeam-teknologi i fødevareindustrien, som funktion af stråleenergi målt i MeV (Pillai & Shayanfar, 2017).

Energy Profile	Food Industry Applications
Low energy (< 1 MeV)	Aseptic packaging, surface sterilization, printing ink curing, food packaging modifications
Medium energy (1 MeV–5 MeV)	Surface sterilization, food pasteurization in customized packaging, packaging polymer modifications
High energy (5 MeV–10 MeV)	Food pasteurization, food sterilization phytosanitary treatment, food industry liquid and solid waste treatment

Resultater af særlig interesse

- Kød:
    - Bruges rutinemæssigt på kyllingefødder, frølår og forskellige andre kødprodukter (Pillai & Shayanfar, 2017)
    - Eliminerede *Salmonella* positive (fra 40% positive) på naturligt kontaminerede fileter af kyllingebryst ved 1 kGy (Lewis et al. 2002)
    - Bestrålingsdoser på 2 og 3 kGy var effektive til at opnå 4-5 logreduktioner i *S. Typhimurium*-populationer på kyllingebryst (Sarjeant et al. 2005)
    - 2-log reduktion af *Salmonella* ved eBeam bestråling (3 kGy) af oksekød og 4-log reduktion ved kombination med mælkesyre (Li et al. 2015)
    - Kombinationer af organisk syre og bestråling var mere effektive til at reducere og bevare lave APC og coliforme niveauer under lagring end hver af behandlingerne alene. Kombinerede behandlinger, der omfatter lavere bestrålingsdoser end dem, der kræves til bestråling alene, kan anvendes til at forlænge holdbarheden af svinekamme under opbevaring efter bestråling uden at øge lipidoxidationen (Kim et al. 2004)

- Fisk:
    - 2,5 log reduktion af *Listeria* i laks ved 1 kGy (Su et al. 2008)

Lovgivning

- EU/øvrigt udland: se afsnit 7.2 og afsnit 11.

Indvirkning på bæredygtighed og miljø

- Energi LAV/MIDDEL – kan være meget energieffektiv (afhængigt af specifik type)
- Vand INGEN – intet vandforbrug
- Kemi INGEN – intet kemikalieforbrug, ingen kemikalierester
- Arbejdsmiljø LAV – on/off teknologi

Krav til effekt

- Tid: få sekunder til minutter
- Afstand: uvist
- Dosis: <5 kGy

Omkostninger

- Anskaffelse: anlæg skal special-bygges i en separat bygning
- Drift: energieffektivt, udgifter til operatører og transport til/fra produktionsområdet

Fordele

- Intet vandforbrug, ingen kemikaliebrug eller -rester, hurtigtvirkende, energieffektivt, kan anvendes på fuldt emballeret produkt, ægte on/off-effekt

Ulemper

- Tab af vitaminer, accelereret lipidoxidation, nedsat pH, ændringer i farve/smag/aroma, forbrugeropfattelse, arbejdsmiljø udfordret, store kapitalinvesteringer

Kildehenvisninger

Ahn, D.U., Kim, I.S & E.J. Lee (2013) Irradiation and additive combinations on the pathogen reduction and quality of poultry meat. Poultry Science 92:534-545.

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7.2.2. Gammastråling

Dokumenteret effekt på fersk kød	*Listeria* reduktion	*Salmonella* reduktion	Holdbarheds-forlængelse
	1,5-5 log	? log	>20 dage[3]

Kødtyper testet	Gris	Okse	Fjerkræ	Andet
	X			RTE, fisk, kanin

(Se detaljer i tabellerne i bilag 2-4 samt Bari et al. 2006)

Resultater af særlig interesse

- Kød
  - Effekt mod mikroorganismer (ikke *Listeria* eller *Salmonella*) påvist i forskellige kødprodukter (se bilag 3 tabel 2.5)
  - En 5-log reduktion af *L. monocytogenes* i hakket grisekød kræver en bestrålingsdosis på a 3.0-kGy, som også holder niveauet af APC og coliforme konstant over en 60-dages periode (Bari et al. 2006)
- Fisk
  - Effekt mod mikroorganismer (ikke *Listeria* eller *Salmonella*) påvist i forskellige fiskeprodukter (se bilag 3 tabel 2.5)

Lovgivning

- EU/øvrigt udland: se afsnit 7.2 og afsnit 11

Indvirkning på bæredygtighed og miljø

- Energi LAV/MIDDEL – kan være meget energieffektiv (afhængigt af specifik type)
- Vand INGEN – intet vandforbrug
- Kemi HØJ – radioaktivt affald
- Arbejdsmiljø HØJ – kræver særlige foranstaltninger og specialuddannet personale

Krav til effekt

- Tid: få sekunder til minutter
- Afstand: uvist
- Dosis: <5 kGy

Omkostninger

- Anskaffelse: afhængig af eksisterende anlæg (Risø i DK)

[3] 3 kGy/0°C (Bari et al. 2006), kombineret med MAP (Pillai & Shayanfar 2017)

- Drift: udgifter til transport mellem produktionssted og behandlingsanlæg, afregning pr. enhed eller kg med serviceudbyder

Fordele

- Intet vandforbrug, ingen kemikaliebrug eller -rester, energieffektivt, kan anvendes på fuldt emballeret produkt, dyb gennemtrængning

Ulemper

- Tab af vitaminer, langsomt virkende (relativt til eBeam og røntgen), accelereret lipidoxidation, nedsat pH, ændringer i farve/smag/aroma, forbrugeropfattelse, udfordret arbejdsmiljø, nationale sikkerhedsudfordringer (radioaktivt materiale), begrænset adgang til radioaktivt materiale og høje omkostninger, store kapitalinvesteringer

Kildehenvisninger

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7.2.3. Røntgenstråling (X-ray)

Dokumenteret effekt på fersk kød	*Listeria* reduktion ? log	*Salmonella* reduktion ? log	Holdbarheds-forlængelse ? dage

Kødtyper testet	Gris	Okse	Fjerkræ	Andet RTE, skaldyr

Resultater af særlig interesse

- Kød
  - 2,8-4,0 log reduktion af *S. Typhimurium*, *E. coli* O157:H7 og *L. monocytogenes* på RTE kogt skinke ved behandling med 0,2 kGy og >6 log reduktion ved behandling med 0,6 kGy (Cho et al. 2019)
- Fisk
  - 4,0 log reduktion af *Salmonella* i rejer ved 1 kGy (Mahmoud 2009)

Lovgivning

- EU/øvrigt udland: se afsnit 7.2 og afsnit 11

Indvirkning på bæredygtighed og miljø

- Energi LAV/MIDDEL – kan være meget energieffektiv (afhængigt af specifik type)
- Vand INGEN – intet vandforbrug
- Kemi INGEN – intet kemikalieforbrug, ingen kemikalierester
- Arbejdsmiljø LAV – on/off teknologi

Krav til effekt

- Tid: få sekunder til minutter
- Afstand: uvist

Omkostninger

- Anskaffelse: kan eksisterende røntgenanlæg benyttes?
- Drift: lavt energiforbrug, operatører er på produktionssteder i forvejen

Fordele

- Intet vandforbrug, ingen kemikaliebrug eller -rester, hurtigtvirkende, kan anvendes på fuldt emballeret produkt, ægte on/off-effekt, bedre gennemtrængningsevne end eBeam

Ulemper

- Tab af vitaminer, accelereret lipidoxidation, nedsat pH, ændringer i farve/smag/aroma, forbrugeropfattelse, arbejdsmiljø udfordret, store kapitalinvesteringer

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7.3. HPP / UHP / HHP

Dokumenteret effekt på fersk kød	*Listeria* reduktion >5 log[4]	*Salmonella* reduktion >5 log[4]	Holdbarheds-forlængelse 6 dage[5]

Kødtyper testet	Gris	Okse	Fjerkræ	Andet
	X		X	RTE

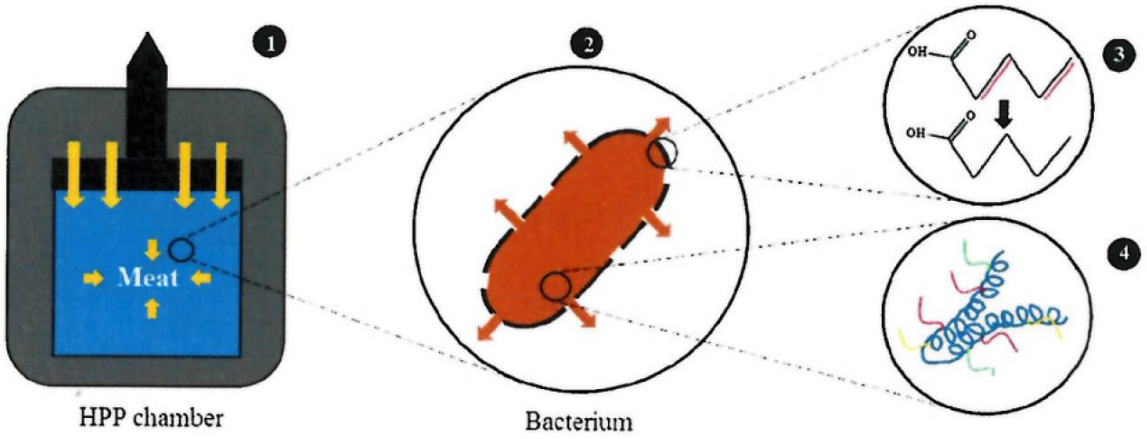
(Se detaljer i tabel 2 i bilag 5)

Virkemåde

Mekanismerne for bakteriel inaktivering er en kombination af faktorer, såsom skade på cellemembranstruktur og modifikation af de cellulære funktioner der er forbundet med protein- eller enzymaktiviteter. Cellemembranforstyrrelse, der forårsager lækage mellem de indre og ydre membraner, er hovedårsagen til celledød. En anden mekanisme, der involverer denaturering (udfoldelse) af proteiner og ændringer i enzymmedierede genetiske mekanismer (figur 7), har også en indflydelse. Delvis proteindenaturering kan opnås med et tryk på omkring 100 MPa, en højere dosis (fx 200 MPa) kan forårsage skade på cellemembranen og strukturerne, og et tryk på omkring 300 MPa forårsager irreversibelt brud på cellemembranen med frigivelse af intracellulære stoffer og deraf følgende celledød. HPP er ikke afhængig af volumen; tryk overføres øjeblikkeligt og ensartet gennem prøven og produktets geometri er irrelevant. Ved forarbejdning af kød pakkes produktet i egnet emballagemateriale, normalt under vakuum, og anbringes i trykkammeret. Kammeret er fyldt med en tryktransmitterende væske (propylenglycol eller

[4] Kombineret med carvacrol (bilag 5, tabel 2)
[5] Ved 4°C

vand). Efter indsættelse af det emballerede produkt lukkes kammeret, tryk påføres i den bestemte tid, tryk frigives, hvorefter produktpakken fjernes.



Figur 7. Den mikrobielle inaktiveringsmekanisme for HPP (Rosario et al. 2021).

Indvirkning på ikke-mikrobielle kvalitetsparametre

Meget høje trykniveauer kan øge temperaturen i produktet og irreversibelt ændre teksturen. Meget højt tryk og/eller lang holdetid kan påvirke oxidation af lipider og proteiner. Påvirkning (denaturering) af enzymstrukturer, der derved påvirker deres funktion, kan have en positiv indvirkning på fordærv. Virkningerne på fysisk-kemiske og sensoriske parametre på en række af forskellige typer fisk, skaldyr, kød og produkter heraf gennemgås i Rosario et al. 2021 (se også bilag 3, tabel 2.6).

Industrielt brug

HPP har fundet anvendelighed i forarbejdningen af en række produktkategorier, herunder kogt kød, skaldyr og fisk, grøntsager og frugtsaft. I 90'erne og 0'erne blev der etableret store HPP-forskningsprogrammer i Japan, Europa og USA. Det er nu blevet en kommercielt implementeret teknologi med omkring 82 kommercielle HPP-systemer til fødevarer i brug over hele verden med en anslået produktion på 150.000 tons/år og salg over 2 milliarder dollars årligt i USA alene. Listen over lande, der forarbejder og sælger højtryksprodukter, vokser også hurtigt; disse omfatter Canada, USA, Mexico, Colombia, Chile, Brasilien, Irland, Storbritannien, Norge, Finland, Polen, Tyskland, Frankrig, Italien, Spanien, Portugal, Indien, Korea, Japan, Australien og New Zealand (Chauhan 2019). Bruges til hos det tyske firma Abraham til dekontaminering af tørrede, saltede skinkeprodukter.

Kommercielle udbydere

- *Hiperbaric* (Spanien) (https://www.hiperbaric.com/) – indenfor kødindustrien sælges udstyr hovedsageligt til produktion af deli meat
- *JBT-Avure Technologies* (US) (https://www.jbtc.com/) – angiver at deres udstyr kan benyttes til dekontaminering af fersk kylling og gris
- *Multivac/Thyssenkrupp Uhde High Pressure Technologies* (Tyskland) (https://www.thyssenkrupp-industrial-solutions.com) – dekontaminering af deli meat

Resultater af særlig interesse

- Kød
    - Effekten på en række forskellige fordærvelsesorganismer og patogener er blevet testet på et stort udvalg af kød- og kødprodukter (fersk og RTE) (se bilag 3, tabel 2.5 og bilag 5, tabel 2)
- Andre fødevarer
    - Effekten på en række forskellige fordærvelsesorganismer og patogener er blevet testet på et stort udvalg af fisk, skaldyr og produkter heraf (se bilag 2 og Rathod et al. 2022)

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området
- EU: ingen specifikt lovgivning, men EFSA har vurderet effekten af HPP-behandling mod bl.a. *L. monocytogenes, Salmonella* og *E. coli* i RTE kød og fundet at metoden er effektiv til at ødelægge skadelige mikroorganismer og ikke giver anledning til flere fødevaresikkerhedsproblemer end andre behandlinger

Indvirkning på bæredygtighed og miljø

- Energi LAV – energieffektivt
- Vand LAV/MIDDEL – afhængigt af hvilken procesvæske der bruges
- Kemi INGEN/MIDDEL – afhængigt af hvilken procesvæske der bruges
- Arbejdsmiljø INGEN/LAV – ved korrekt installation

Krav til effekt

- Tid: ca. 10-15 minutter
- Afstand: ikke relevant

Omkostninger

- Anskaffelse: behovet for trådvikling (for sikker og pålidelig drift) og det begrænsede udbud af trykbeholdere, der kan operere over 680 MPa øger udstyrsomkostningerne

- Drift: driftsomkostningerne ved produkter forarbejdet med HPP er reduceret betydeligt i de senere år – de gennemsnitlige omkostninger ved HPP-behandling af fx RTE kød, er anslået til at ligge omkring 0,50-2,00 DKK/kg (lavere end omkostningerne for varmebehandling?)

Fordele

- Hurtigtvirkende, energieffektivt, uafhængigt af form/størrelse af produktet, bruges på emballeret produkt, kan deaktivere enzymer (der faciliterer ikke-mikrobielt fordærv)

Ulemper

- Risiko for ændringer i farve/tekstur og lipid oxidation, ændrer struktur af proteiner/enzymer, kan forårsage 'kogt' udseende, ingen effekt på sporer, risiko for at kompromittere emballagens integritet, store kapitalinvesteringer

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7.4. Klor / klorholdige produkter

Dokumenteret effekt på fersk kød	*Listeria* reduktion 1-3 log	*Salmonella* reduktion 1-3 log	Holdbarheds-forlængelse ? dage

Kødtyper testet	Gris	Okse	Fjerkræ	Andet
	X	X	X	RTE, fisk, skaldyr

(Se detaljer i Anonymous 2009)

Virkemåde

Behandling med klor forårsager lækage af makromolekyler fra bakteriecellerne, hvilket indikerer permeabilitetsændringer i membranen. Den antimikrobielle virkning af klor afhænger hovedsageligt af mængden af frit klor (HOCl), der er til stede i vandet (også kaldet 'available chlorine concentration' (ACC)), vandtemperatur, kontakttid og pH.

Elektrolyseret vand (EW), også kendt som ECA- eller ECO-vand, genereres ved elektrolyse af en fortyndet salt (NaCl) opløsning, der danner en opløsning af hypochlorsyre (HOCl) og natriumhydroxid (NaOH). Elektrolysen producerer tre typer EW (tabel 1). Sur EW (AEW), med pH 2-3 og redox-potentiale (ORP) >1000 mV, produceres på anode siden. Basisk EW (BEW), med pH 10-13 og ORP -800 til -900 mV, genereres på katodesiden. Neutral EW (NEW), med pH 6,5-7,5 og ORP 700 til 800 mV, kan fremstilles på forskellige måder; det kan genereres, når elektrolytcellen ikke har en separativ membran eller ved at blande katolytten med en fortyndet NaCl-opløsning. Det er blevet rapporteret, at NEW er mere stabil og holder sin antibakterielle aktivitet bedre under opbevaring sammenlignet med AEW og BEW.

Tabel 6. Egenskaber for forskellige typer elektrolyseret vand (Ramirez Orejel et al. 2020).

Type elektrolyseret vand	pH	ORP (mV)
Sur (AEW)	2-3	>1000
Basisk (BEW)	10-13	-800 til -900
Neutral (NEW)	6,5-7,5	700 til 800

Den antimikrobielle virkning af EW er baseret på den kombinerede virkning af pH, ORP og ACC.

De mekanismer, hvormed klor og EW forårsager mikrobiel inaktivering, er en kombination af faktorer, herunder skade og nedbrydning af cellevægsstrukturer, forstyrrelse af cellemembranpermeabilitet, denaturering og inaktivering af proteiner samt beskadigelse af nukleinsyrerne RNA og DNA.

Indvirkning på ikke-mikrobielle kvalitetsparametre

Brug af klorforbindelser i høje koncentrationer kan have en uønsket virkning på sensoriske parametre. Produktet kan i det tilfælde efterlades med en lugt af klor og gulfarvning af kød er også blevet observeret.

Industrielt brug

Klor bruges rutinemæssigt til produktdekontaminering af grøntsager, fjerkræ og skaldyrsprodukter i mange lande.

Resultater af særlig interesse

- En undersøgelse udført på svinekam viste, at EW'er (AEW og BEW) ikke havde nogen effekt på lipidoxidation, at AEW øgede proteinoxidation, mens BEW reducerede oxidationsreaktionerne (Athayde et al. 2017).

- En ekspertkomite (FAO/WHO) i 2009 fandt ingen grund til sundhedsmæssige betænkeligheder, så længe klor-residualer på/i produkterne efter behandling påviseligt er minimale (Anonymous 2009)

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området

Indvirkning på bæredygtighed og miljø

- Energi LAV – energieffektivt
- Vand MIDDEL – behov for vand til at lave brugsopløsning
- Kemi MIDDEL/HØJ – forbrug af enten klor eller NaCl, klor i afløbsvand
- Arbejdsmiljø MIDDEL/HØJ – luftvejsirritation, risiko for dannelse af klorgas

Krav til effekt

- Tid: ca. 5-30 sek (afhængigt af pH og temperatur)
- Afstand: kontakt

Omkostninger

- Anskaffelse: kommerciel sprayer (klor), elektrolyse generator (EW)
- Drift: klor eller NaCl

Fordele

- Lave driftsomkostninger, lavteknologisk (klor), generering på stedet (EW), lille kapitalinvestering

Ulemper

- Indvirkningstid flere minutter, mulige sensoriske defekter på produkter, risiko for lipid- og proteinoxidation, arbejdsmiljø (lugt), korrosion af udstyr

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7.5. Kold plasma / NTAP

Dokumenteret effekt på fersk kød	*Listeria* reduktion >1 log	*Salmonella* reduktion >1 log	Holdbarheds-forlængelse ? dage

Kødtype testet	Gris	Okse	Fjerkræ	Andet
	X	X	X	RTE

(Se detaljer i tabel 3 i bilag 5)

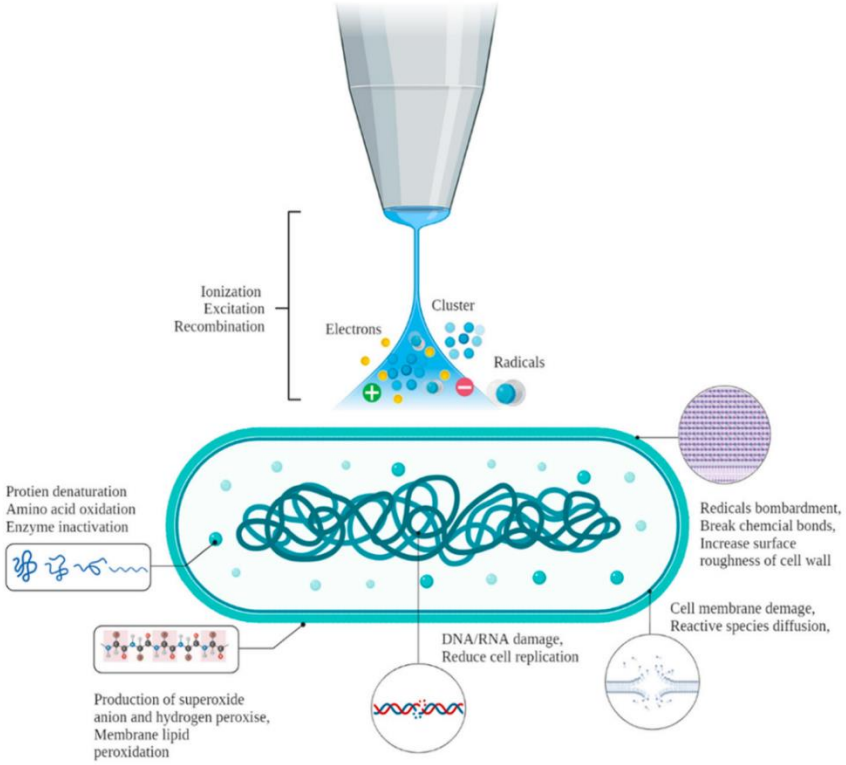
Virkemåde

Kold plasma (cold plasma, CP), også kaldet Non-Thermal Atmospheric Plasma (NTAP), genereres under atmosfæriske forhold ved temperaturer på 30-60°C, og kræver ikke meget energi. CP fremstilles ved at fremføre fødegas (atmosfærisk luft eller en hvilken som helst gasblanding) mellem to elektroder for at generere et kraftigt elektrisk felt. Dette kan opnås enten ved hjælp af dielektrisk barriereudladning (dielectric barrier discharge, DBD), radiofrekvens eller mikrobølgestrømkilder. De ioniserede gasser der dannes, består af kemisk reaktive arter, såsom positive og negative ioner, radikaler, elektroner, exciterede og neutrale molekyler, ultraviolette fotoner og synligt lys. Selvom den nøjagtige mekanisme for den antibakterielle virkning endnu ikke er fuldt belyst, ved man at CP er kilde til flere kemisk reaktive arter, herunder reaktiv ilt (ROS) og reaktive nitrogenarter (RNS), der er stærkt antimikrobielle (se figur 8). Rapporterede virkningsmekanismer er dannelsen af ladede partikler, reaktive oxidantarter, ozon (hvis behandling udføres i et ilt-/luftmiljø) og UV-stråling. Sammensætningen af den fødegas, der anvendes til at generere CP, påvirker typen af reaktive arter, f.eks. genererer ilt (O_2) O, OH-, H_2O_2 og O_3, mens nitrogen (N_2) genererer NO, NO_2, N_2O_3 og N_2O_5.

NTAP kan genereres ved hjælp af atmosfærisk trykplasmastråle (APPJ), som har både teknisk og kommercielt mere økonomisk interesse for fødevareindustrien, da det fungerer under almindelige produktionsforhold. Temperaturen af dissocierede partikler forbliver relativt kold i NTAP, fordi det meste af den

elektriske energi kanaliseres til elektronkomponenterne uden at opvarme hele gasdampen. I fødevareforarbejdningsindustrien kan anvendelse af energiske, reaktive gasser af CP på overflader af fødevarer, såsom frugt, grøntsager, kød og fjerkræ osv. effektivt reducere den mikrobielle belastning.

Dielektrisk barriereudladningsplasma (DBD-plasma): Denne type system er baseret på et særligt AC-udledningsanlæg, der genererer plasma under atmosfærisk tryk ved moderat gastemperatur. DBD er også kendt som barriereudladning eller lydløs udladning. DBD-plasma anvendes generelt til fx overfladedekontaminering, sterilisering, ozongenerering, forureningskontrol, kemisk dampaflejring og overfladeaktivering. En forholdsvis nyudviklet teknologi er et DBD-plasma system til emballerede produkter pakket med en specifik gassammensætning (patenteret af Bindslev og Leipold i 2009). I dette system føres det emballerede produkt mellem to elektroder, og det plasma der genereres inde i pakken, hæmmer/inaktiverer mikrober.



Figur 8. Effekt af CP-behandling på en bakteriecelle (Roobab et al. 2022).

Indvirkning på ikke-mikrobielle kvalitetsparametre

Nogle af de reaktive stoffer, der genereres i processen, er kendt for at fremkalde oxidation. Dette kan påvirke flygtige forbindelser, lipider og proteiner afhængigt af den anvendte fødegas, applikationsteknologien og procestiden. Nogle oxidative virkninger kan minimeres ved at kombinere CP med andre interventioner, f.eks. planteekstrakter (se bilag 5, tabel 3). Nogle forskergrupper har observeret en stigning i

overfladetemperaturen på produkterne, og procesparametrene skal derfor vælges med omhu og overvåges nøje. Modifikation (denaturering) af enzymer, der derved påvirker deres funktion, kan have en positiv indvirkning på ikke-mikrobielt fordærv.

Industrielt brug

Det har ikke været muligt at finde information om brug af CP i større industriel skala.

Resultater af særlig interesse

- Kød
    - Effekt er påvist på fersk kylling, gris og okse såvel som forskellige RTE-produkter (se bilag 5, tabel 3)

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området

Indvirkning på bæredygtighed og miljø

- Energi LAV – minimalt behov for energi til at generere CP
- Vand INGEN
- Kemi LAV/MIDDEL – afhænger af hvilken type fødegas der bruges
- Arbejdsmiljø UVIST

Krav til effekt

- Tid: ca. 2-5 min
- Afstand: ca. 2 cm

Omkostninger

- Anskaffelse: uvist, da udstyr i større skala så vidt vides ikke eksisterer endnu
- Drift: afhænger meget af valg af fødegas

Fordele

- Meget omkostningseffektiv (afhænger dog af typen af fødegas), kan bruges på emballerede produkter (en fordel at kombinere med MAP)

Ulemper

- Relativt langsom effekt (minutter), risiko for ændringer i overfladetopografi, off-flavors og off-odors kan dannes under opbevaring, begrænset effekt i fødevarer, mulig toksicitet af de kemiske biprodukter der dannes, behov for udstyr i større skala

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7.6. Ozon / ozoneret vand

Dokumenteret effekt på fersk kød	*Listeria* reduktion 1-6 log	*Salmonella* reduktion >1 log	Holdbarheds-forlængelse 2-6 dage

Kødtype testet	Gris	Okse	Fjerkræ	Andet
		X	X	RTE, fisk, skaldyr

(Se detaljer i bilag 2, i tabel 1 i bilag 5 samt Pandiselvam et. al. 2019)

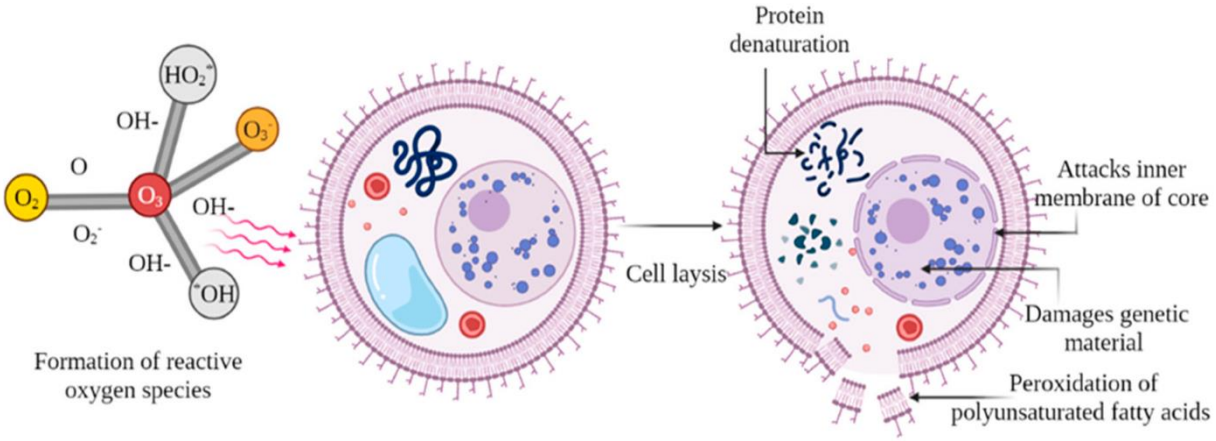
Virkemåde
Ozon er et kraftigt oxidationsmiddel. Det er et mere effektivt desinfektionsmiddel end både klor og klordioxid. Det ødelægger bakterier, gær, skimmelsvampe og parasitter og er mest effektivt ved pH 6,0 - 8,5.

Der er foreslået to mekanismer til at forklare den antimikrobielle virkning af ozon: direkte interaktion mellem molekylær ozon (O_3) og vandige systemkomponenter (første ordens reaktioner med højt

redoxpotentiale) og/eller antimikrobiel aktivitet medieret af frie radikaler. Virkningen af ozon på mikroorganismer afhænger af, hvor meget organisk materiale der omgiver bakteriecellerne. Nogle sporer er resistente over for ozon, mens andre er følsomme. Inaktivering af mikroorganismer hævdes at forekomme på grund af beskadigelse eller permanent opløsning af cellemembranen, som fører til efterfølgende lækage af intracellulære indholdsstoffer og celledød (se figur 9).

Ozon har en stærk karakteristisk lugt, er en ustabil gas ved stuetemperatur (nedbrydes hurtigt) og er mere opløselig i koldt vand end i varmt vand. Ozon oxiderer kraftigt og direkte de cytoplasmatiske membraner og cellevægge på bakterier. Den antimikrobielle virkning af ozoneret vand finder sted inden for 5 sekunder efter behandlingen. Ved behandling med ozoneret vand, er ozonens antimikrobielle aktivitet ca. 3.000 gange højere end den, der observeres med klor (Pandiselvam et al. 2019). Ozoneret vand er blevet effektivt udnyttet til at fjerne eller inaktivere *E. faecalis, E. coli*, *B. cereus*, *L. monocytogenes*, *S. aureus* og *Y. enterocolitica*.

Effektiviteten afhænger i høj grad af valget af en tilstrækkeligt effektiv ozondosis. Doseringsbestemmelse er en fin balance mellem at være tilstrækkelig til at inaktivere målorganismerne men samtidigt undgå at påvirke kvalitetsparametre i produktet. Høje niveauer af ozon kan have en negativ effekt på produktets kvalitetsparametre såsom reduktion af polyfenoler, vitaminer og flygtige forbindelser (fx aromastoffer) og kan have en negativ indvirkning på fasthed og farve.



Figur 9. Skematisk præsentation af dekontaminering ved brug af ozon (Roobab et al. 2022).

Indvirkning på ikke-mikrobielle kvalitetsparametre

Hvis det er nødvendigt at benytte høje koncentrationer af ozon (enten på gasform eller i vandig opløsning) for at få den ønskede bakteriedræbende effekt, kan det påvirke produktets ikke-mikrobielle kvaliteter negativt (fx vitaminer, flygtige forbindelser, farve og fasthed). På den anden side kan nogle af disse virkninger være ønskelige for forbrugernes accept af produktet. Ozon bruges i vid udstrækning til at

fjerne lugt, og det har vist sig at kunne fjerne off-odor forbindelser, såsom geosimin i fisk samt skatol-, indol- og svovlholdige forbindelser ('hangriselugt') i svin.

Industrielt brug

- Bruges over hele verden til at desinficere drikkevand. Ozongeneratorers kapacitet varierer afhængigt af applikationen. Store enheder behandler mere end 100.000 liter/time i kommunale vandforsyninger. Små bordmodeller behandler 1.500 liter/time i laboratorier og fødevareforarbejdningsanlæg (til fx skærebrætter, redskaber og hånddesinfektion)
- Bruges til at dekontaminere rugeæg. På DanHatch bliver rugeæg placeret i et rum med 1 ppm ozon i 5-6 timer, for at forebygge krydskontaminering af *Salmonella* fra æg til kylling.

Kommercielle udbydere

- Jimco A/S (DK) https://jimco.dk/ - udstyr til kombineret behandling med ozon og UV-C
- Absolute Ozone (Canada) https://absoluteozone.com/ozone-applications/food-processing-storage/ozone-for-meat-poultry-fish-disinfection/ - eksempel på slagtekroppe behandlet med ozoneret vand.
- Ozone Solutions (US) https://ozonesolutions.com/ - ozonkamre, standard og specialbyggede
- Evergreen Techno Plant (Italien) (https://www.etpsrl.eu/?lang=en#!/up)

Resultater af særlig interesse

- Kød
    - Forskning har vist bakteriedræbende effekt overfor *Pseudomonas*, *Enterococci*, *Salmonella*, *Listeria* og *Bacillus* (Pandiselvam et al. 2019)
    - Ozon inaktiverede 2 x 10^6 CFU/g *Listeria monocytogenes* på ferske kyllingeprodukter (Pandiselvam et al. 2019)
    - Ozon reducerede coliforme, aerobe, og anaerobe bakterier med 1,5-2,5 log på kyllingebrystkød og forlængede holdbarheden af kyllingelår med 6 dage (Roobab et al. 2022) (se bilag 5, tabel 1)
    - Det er vist at ozon kan reducere mikrobielle kimtal og forlænge holdbarheden af okse-, gris- og fjerkræprodukter (se bilag 5, tabel 1)
- Fisk
    - Effekten på fordærverorganismer er testet på fisk og skaldyr, hvor der blev opnået log reduktioner på mellem 0,29 og 1,5 (se Andoni et al. 2021) samt en forlængelse af holdbarheden (se Brodowska et al. 2018)
    - Eliminering af geosmin i muskelvævet på fisk ved hjælp af en vaskebehandling med ozon er vist effektiv (Pandiselvam et al. 2019)

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området

- USA: FDA har godkendt brugen af ozon som antimikrobielt middel til direkte kontakt med alle fødevarer (26. juni 2001). USA's landbrugsministerium har accepteret ozon som et antimikrobielt middel til direkte kontakt med kød, fjerkræ, fisk, bløddyr og krebsdyr (december 2001). Ozon har haft USDA-GRAS status siden 2002 til desinfektion af kød, fjerkræ og æggeprodukter (FSIS directive 7120.1 rev 12).
- Japan: Japans regering tillod i 1996 anvendelse af ozon i direkte kontakt med alle typer fødevarer.
- Canada: Det canadiske fødevareinspektionsagentur (CFIA) har godkendt brugen af ozon til rengøring af fødevarekontaktflader.
- Australien: Australiens regering godkendte i 1996 brugen af ozon til kontakt med alle fødevarer.

Indvirkning på bæredygtighed og miljø

- Energi LAV – energi nødvendig til at lave ozon/ozoneret vand
- Vand LAV/MIDDEL – mere hvis der bruges ozoneret vand
- Kemi LAV – ingen reststoffer
- Arbejdsmiljø MIDDEL/HØJ – evt. lugtgener, mistanke om kræftfremkaldende stoffer

Krav til effekt

- Tid: teoretisk indenfor 5-10 sek, men reelt nok nærmere 10 min
- Afstand: kontakt

Omkostninger

- Anskaffelse: uvist
- Drift: uvist

Fordele

- Ingen kemiske reststoffer, måske hurtigtvirkende, holdbarhedsforlængende, minimal ændring i ernæringsmæssige, kemiske og fysiske egenskaber, forbedring af sensoriske kvaliteter, kan bruges på både friske og frosne fødevarer, lave driftsomkostninger

Ulemper

- Genererer farlige oxidanter, bekymringer vedrørende kræftfremkaldende biprodukter, effekt på forskellige mikroorganismer varierer betydeligt, svær balance mellem bakteriedræbende effektivitet og skadelig virkning på kvalitetsparametre, udfordringer med arbejdsmiljø, høje kapitalinvesteringer

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7.7. Pulsed Electric Field (PEF)

Dokumenteret effekt på fersk kød	*Listeria* reduktion ? log	*Salmonella* reduktion ? log	Holdbarheds-forlængelse ? dage

Kødtype testet	Gris	Okse	Fjerkræ X	Andet Fisk, skaldyr

(Se detaljer i Chauhan 2019 og Rathod et al. 2022)

Virkemåde

Pulsed Electric Field (PEF) behandling er en ikke-termisk dekontamineringsteknik, der bruger korte impulser af elektricitet til at inaktivere mikroorganismer ved irreversible strukturelle ændringer i

membranen, hvilket resulterer i poredannelse og tab af membranens selektive permeabilitetsegenskaber. Omfanget af permeabilitetsforøgelsen afhænger af styrken og varigheden af pulsen i det elektriske felt. Når det elektriske felt aktiveres, sker en polarisering og efterfølgende ophobning af frie ladninger på begge sider af celleoverfladen, hvilket fører til en øget transmembranpotentialeforskel og en reduktion af membrantykkelsen, hvilket til sidst resulterer i poredannelse. Effekten af behandlingen afhænger af styrken af det elektriske felt, behandlingsvarigheden og antallet af impulser.

PEF er en metode, der bruger elektriske bølger med højspændingsamplitude. Produktet placeres mellem elektroder i et kammer, og udsættes for korte elektriske impulser (af varighed fra mikrosekunder til millisekunder) af højspænding (typisk 10-80 kV/cm). Afhængigt af produktets sammensætning og de virkninger, der skal opnås, kan procesbetingelserne såsom styrken af det elektriske felt (kV/cm), pulsfrekvens, pulsbredde, pulsbølgens form og eksponeringstid (relateret til strømningshastigheden og væskevolumenet i elektrodekammeret) justeres på passende vis. For eksempel forårsager en styrke af det elektriske felt i intervallet 0,1-1 kV/cm en reversibel permeabilisering af planteceller, 0,5-3 kV/cm resulterer i en irreversibel permeabilisering af plante- og animalsk væv, og en feltstyrke på 15-40 kV/cm giver en irreversibel permeabilisering af mikrobielle celler.

PEF bruger meget lidt energi til at generere de elektriske impulser, men energien fra de elektriske impulser genererer varme på grund af Joule-opvarmning, så kontinuert afkøling er nødvendig for at opretholde en lav temperatur på det forarbejdede produkt under PEF-behandling.

Indvirkning på ikke-mikrobielle kvalitetsparametre

PEF har minimal skadelig virkning på fødevarekvalitetsegenskaber, såsom termolabile komponenter, vitaminer, smagsstoffer og aromaer. Ved meget højt tryk eller langvarig anvendelse kan PEF danne huller i muskelfibrene.

Industrielt brug

De største leverandører af udstyr i kommerciel skala er Elea (Quakenbruck, Tyskland) og Pulsemaster (Ladel, Nederlandene), der har specialiseret sig i PEF-behandling med henblik på mild konservering. Den nuværende udformning af trykkamre tillader ikke forarbejdning af faste fødevarer og begrænser dermed dens anvendelse til en lang række produkter. Høje kapitalomkostninger ved udstyret er en anden barriere, der har hæmmet kommercialiseringen af denne teknologi. I den nuværende udformning af udstyret skal elektroderne udskiftes efter ca. 100 timers drift. Forbedring af elektrodepålidelighed og optimeret design af trykkamre er spørgsmål, der skal håndteres før PEF som desinficeringsteknik kan vinde udbredelse.

Kommercielle udbydere:

- Pulsemaster (US, Holland, Tyskland) (https://www.pulsemaster.us/)

Resultater af særlig interesse

- Kød
    - Ifølge Meat & Livestock Australia, har PEF som dekontamineringsmetode begrænset anvendelighed på faste fødevarer såsom kød og kødprodukter pga. lav ledningsevne og højt protein- og fedtindhold.
- Fisk
    - Det er vist at PEF kan reducere niveauerne af aerobe kimtal, psykrofile kimtal, *Pseudomonas*, *Enterobacteriaceae* og H_2S-producerende bakterier i fisk og skaldyrsprodukter (se Rathod et al. 2022)

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området
- EU: PEF betragtes stadig som en ny teknologi. I EU findes der ingen særlig lovgivning om fødevarer, der er forarbejdet med PEF. Generelt er anvendelsen af denne teknik reguleret af forordningen om 'nye fødevarer' (EU) 2015/2283, men anvendelsen af PEF i produktionen betyder ikke automatisk, at fødevaren kategoriseres som en 'ny fødevare'. I henhold til artikel 4 i forordning (EF) nr. 258/97 kan en fødevare betragtes som 'ny fødevare', hvis den anvendte produktionsproces medfører væsentlige ændringer i dets sammensætning eller struktur og påvirker næringsværdien, metabolismen eller niveauet af uønskede stoffer. Anvendelsen af nye forarbejdningsteknologier har potentiale til at mindske fødevareproduktionens miljøpåvirkning og øge fødevaresikkerheden, så deres anvendelse fremmes lovgivningsmæssigt i EU ((EU) 2015/2283).
- USA: Før 2002 definerede FDA pasteurisering som en varmebehandling, men i september 2004 omdefinerede USDA National Advisory Committee on Microbiological Criteria for Foods (NACMCF) udtrykket 'pasteurisering' til at betyde 'enhver proces, behandling eller kombination heraf, der anvendes på fødevarer for at reducere de fleste mikroorganismer af folkesundhedsmæssig betydning til et niveau, der sandsynligvis ikke vil udgøre en folkesundhedsrisiko under normale forhold for distribution og opbevaring'. Denne omdefinering gør det muligt at anvende metoder som fx PEF til dekontaminering af fødevarer.
- Andre lande: der findes regler tilsvarende EU's, vedrørende 'nye fødevarer' i Canada, New Zealand/Australien, Kina og Brasilien, men definitionen af en 'ny fødevare' kan variere.

Indvirkning på bæredygtighed og miljø

- Energi MIDDEL – behov for kontinuerlig afkøling af produktet
- Vand INGEN
- Kemi INGEN
- Arbejdsmiljø UVIST

Krav til effekt

- Tid: få sekunder
- Afstand: uvist

Omkostninger

- Anskaffelse: uvist, da udstyr i større skala til faste produkter så vidt vides ikke eksisterer endnu, men formodentlig en betragtelig investering
- Drift: uvist, da udstyr i større skala til faste produkter så vidt vides ikke eksisterer endnu men udgifter til nedkøling af produkt må påregnes

Fordele

- Hurtigtvirkende, meget skånsom overfor ikke-mikrobielle kvalitetsparametre, holdbarhedsforlængende effekt bevist (patenteret i 1960), ingen brug af vand eller kemikalier

Ulemper

- Ingen effekt mod sporer, uhensigtsmæssig effekt overfor ikke-mikrobielle kvalitetsparametre ved høj strømstyrke og/eller lang behandlingstid, effekt er pH-afhængig, risiko for korrosion af elektroder af forurening af produktet med korroderede affaldsstoffer, store kapitalinvesteringer

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7.8. Ultralyd

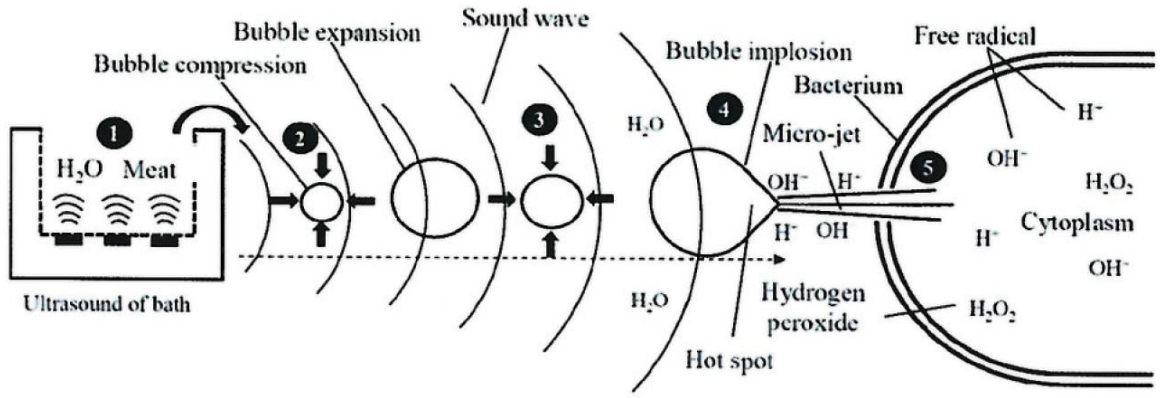
Dokumenteret effekt på fersk kød	*Listeria* reduktion	*Salmonella* reduktion	Holdbarheds-forlængelse
	1 log	1-2 log	? dage

Kødtype testet	Gris	Okse	Fjerkræ	Andet
	X	X	X	Fisk

(Se detaljer i tabel 2.5 i bilag 3 samt i bilag 6)

Virkemåde

Ultralydsudstyr producerer lydbølger, der forplanter sig i et flydende medium (eller i fødevarematricen). Spredningen af bølgerne skaber områder med undertryk, der ændrer væske til dampfasen og danner små bobler. Efter successive kompressioner og udvidelser imploderer boblerne og genererer mikrojet-stråler, frie radikaler, hydrogenperoxid og hot spots - alle faktorer der forårsager mikrobiel celledød (se figur 10).



Figur 10. Ultralyds mikrobielle inaktiveringsmekanismer (Rosario et al. 2021).

Indvirkning på ikke-mikrobielle kvalitetsparametre

Meget kraftige mikrojet-stråler kan generere en forskydningskraft, der har kapacitet til ikke blot at påvirke mikrobielle celler, men også dyre- og planteceller.

Industrielt brug

- Kommerciel anvendelse af ultralyd har indtil for nyligt været hæmmet af høje omkostninger for anskaffelse af udstyr og et højt energiforbrug. Producenter af ultralydsudstyr har imidlertid

fokuseret på at udvikle systemer til at reducere driftsomkostningerne ved at designe flere systemer i serie og parallelt, der muliggør større strømningshastigheder. Moderne udstyr har et energiforbrug pr. liter/kg behandlet materiale der er sammenligneligt med enhver anden enhedsoperation i branchen, er robust og holdbart hvor kun sonder, der er i direkte kontakt, kræver udskiftning hver 18. måned. Bruges i kombination med damp til dekontaminering af fjerkræslagtekroppe hos bl.a. Faccenda Foods og Cargill (UK).

Kommercielle udbydere

- SANOVO Process SonoSteam (DK); (https://www.sanovogroup.com)
  Dekontaminering af patogener på overfladen af slagtekroppe af fjerkræ ved kombination af damp og ultralyd via specialdesignede dyser.

Resultater af særlig interesse

- Kød og fisk
  - 2,0-2,5 log reduktion af *E. coli*, *S. typhimurium* og *Y. enterocolitica* på fersk grisekød ved en behandling med en kombination af ultralyd (2 sek) og damp (Turantas et al. 2015 og bilag 6)
  - Beskedne reduktioner på forskellige kød- og fiskeprodukter og mere udtalt effekt i kødmodelsystemer (se bilag 3, tabel 2.5)

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området

Indvirkning på bæredygtighed og miljø

- Energi MIDDEL
- Vand LAV
- Kemi INGEN
- Arbejdsmiljø LAV

Krav til effekt

- Tid: få sekunder – bedst i kombination med andre metoder
- Afstand: kontakt

Omkostninger

- Anskaffelse: uvist, da udstyr i større skala til faste produkter så vidt vides ikke eksisterer endnu, men formodentlig en betragtelig investering
- Drift: moderat energiforbrug – sammenligneligt med andre industrielle processer

Fordele

- Minimal påvirkning af smag, farve og ernæringsmæssig værdi, kan øge effekten af andre ikke-termiske teknologier (synergi), ingen brug af kemikalier

Ulemper

- Kan forårsage uønskede ændringer af tekstur, de fleste studier er foretaget på laboratorieskala, relativt kompleks teknologi, udstyr i stor skala endnu ikke udviklet til faste fødevarer

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7.9. UV-C lys / pulserende lys

Dokumenteret effekt på fersk kød	*Listeria* reduktion	*Salmonella* reduktion	Holdbarheds-forlængelse
	? log	0,4-3 log	? dage

Kødtype testet	Gris	Okse	Fjerkræ	Andet
	X	X	X	RTE, fisk, skaldyr

(Se detaljer i bilag 2, tabel 2.5 i bilag 3, bilag 7 samt Mahendran et al. 2019)

Virkemåde

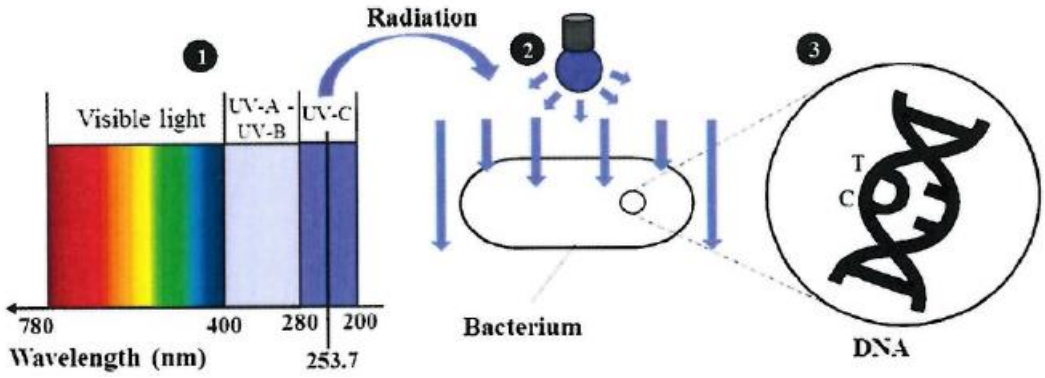
UV-C genereres af lavtryks-kviksølvlamper, og den udsendte stråling (200 - 280 nm, optimum ved 253,7 nm) har vist sig at være effektiv ved mikrobiel inaktivering. Effekten er direkte afhængig af tid og afstand mellem lyskilde og overflade.

UV-C stråler kan trænge ind i bakteriecellen, 'sammensmelte' cellens DNA og RNA ved at indføje en binding mellem thymin (T) og cytosin (C) som ødelægger cellens evne til at replicere/transskribere og dermed forhindrer mikroorganismens vækst (se fig. 11).

UV-C bestrålingens effekt på mikroorganismer er uafhængig af pH, temperatur og matrix. Det vigtige er, at UV-C strålerne kan ramme organismen og betyder, at hvis en bakterie er skjult for UV-lyset, kan den undgå bestråling (såkaldt 'skyggeeffekt'). Der er derfor afgjort bedst effekt på glatte overflader.

UV-lyset kan bruges som eneste desinfektionsløsning, men kan også kombineres med andre teknikker.

Pulserende lys er en videreudvikling af UV-C lys. Ved at pulsere lys i intervallet 185 – 1100 nm, opnås en eksplosiv effekt på cellerne (forhøjet varme og tryk) oven i den effekt der er fra UV-C strålerne alene (se ovenfor).



Figur 11. Den mikrobielle inaktiveringsmekanisme for UV-C bestråling (Rosario et al. 2021).

Indvirkning på ikke-mikrobielle kvalitetsparametre

UV-lys kan nedbryde vitaminer (især A, C, B_2) ved fotonedbrydning. Peroxider produceret ved UV-lys eksponering kan angribe fedtopløselige vitaminer og farvede forbindelser som kan føre til ændring i ernæringsmæssig kvalitet og misfarvning. Desuden kan lange behandlinger med UV-lys øge temperaturen på fødevareproduktet, hvilket vil føre til temperaturrelaterede kvalitetsændringer såsom kogt smag og farveændring på grund af ikke-enzymatisk bruning. UV-lys kan virke oxiderende på lipider (harskning). Under normalt brug af UV- eller pulserende lys vil der pga. den hurtigtvirkende antimikrobielle effekt ikke ske nævneværdige forandringer i de ikke-mikrobielle egenskaber.

Industrielt brug

UV-teknologi bruges over hele verden til at desinficere drikkevand. I USA alene er der mere end 500 UV-anlæg til desinfektion af drikkevand og i Europa er der mere end 2000 anlæg, der benytter sig af denne teknologi.

Selvom pulserende lys ikke er meget udbredt i fødevareindustrien, er det med succes blevet brugt i storstilet kommerciel anvendelse til DVD/CD/Blu-ray-limning og dekontaminering af flaskekapsler. Kommercielt dekontamineringssystem til fødevareemballage baseret på pulserende lys blev fremstillet på foranledning af FDA tilbage i 1995. Derfor kan disse avancerede, store kommercielle systemer let tilpasses fødevareindustrien. Pulserende lys teknologi kan også nemt integreres i eksisterende fødevareforarbejdningslinjer.

Resultater af særlig interesse

- Kød
    - 0,4-1,6 log reduktion af *S. typhimurium* på fersk grisekød ved behandlingstid på 1-30 sek og på svær, en 3-log reduktion ved behandlingstid på 30 sek (Mahendran et al. 2019)
    - Generelt opnås en reduktion på 2 log. Der synes at være meget lille forskel i følsomhed mellem forskellige bakterie-, gær- og skimmelarter (Tomasevic et al. 2019)
- Fisk
    - Effekten af UV-C og/eller pulserende lys på forskellige mikroorganismer er blevet demonstreret på diverse fisk og fiskeprodukter (se bilag 2; bilag 3, tabel 2.5; bilag 7)
    - UV-C doser fra 0,30 til 0,79 J/cm2 hæmmede bakterievækst og den samlede proteinnedbrydning under køleopbevaring af fersk fisk, og forlængede holdbarheden med op til 6 dage. Denne dosis accelererede imidlertid også oxidativ nedbrydning på grund af dannelsen af frie radikaler. Kombination af UV-C med ikke-termiske teknologier der fjerner frie radikaler, kan afhjælpe problemet (Monteiro et el. 2021)

Lovgivning

- Se afsnit 11 for generelle betragtninger vedrørende EU-lovgivning på området
- EU: Fødevarer behandlet med UV-C betragtes som en 'novel food' (og reguleres som sådan), hvis behandlingen ændrer produktet væsentligt. EFSA godkender brugen fra sag til sag. Hvis fødevaren ikke betragtes som en 'novel food', skal producenten følge de generelle og specifikke bestemmelser vedrørende fødevarehygiejne (EF nr. 852/2004) og fødevaresikkerhed (EF nr. 178/2002). Så længe UV-C-bølgelængden er over 100 nm, falder behandlingen ikke ind under direktiv 1999/2/EF for bestråling af fødevarer (DG SANTE via Karsten Snitkjær, NATDIS, e-mail-korrespondance).
- USA: I 1997, godkendte FDA brug af UV bestråling som en alternativ metode til mikrobiel kontrol i kødprodukter. Pulserende lys blev godkendt af FDA i 2002.

Indvirkning på bæredygtighed og miljø

- Energi LAV/MIDDEL – pulserende lys har meget lavt energiforbrug, muligt behov for let nedkøling af produkt
- Vand INGEN
- Kemi INGEN
- Arbejdsmiljø LAV/MIDDEL – ozonudvikling, risiko for skadelige UV stråler

Krav til effekt

- Tid: op til ca. 30 sek
- Afstand: så tæt som muligt

Omkostninger

- Anskaffelse: relativt dyrt udstyr (især pulserende lys)
- Drift: lavt energiforbrug, UV-lampe skal udskiftes jævnligt

Fordele

- Hurtigtvirkende, ingen giftige reststoffer, meget lavt energiforbrug (pulserende lys), lave drifts-omkostninger (UV-C), generel accept fra forbrugere, kan bruges på emballerede fødevarer

Ulemper

- Mulig lipidoxidation og farveændring, skyggeeffekt, begrænset gennemtrængningsevne, op-varmning af fødevaren, ingen effekt på sporer (UV lys), visse stammer er modstandsdygtige (pul-serende lys), store kapitalinvesteringer (pulserende lys)

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8. International perspektivering

For at få et internationalt perspektiv på problemstillingen, blev eksperter fra USA, Storbritannien, New Zealand og Australien kontaktet og spurgt hvad deres umiddelbare holdning ville være vedrørende udsigten til at producere patogenfrit kød og om de havde nogle erfaringer at dele fra deres respektive hjørner af verden. Det resulterede i de følgende betragtninger, som alle har indgået i de samlede overvejelser.

Input fra Larry Keener | International Product Safety Consultants | The Food Safety Professionals | Seattle, WA, USA

I assume that the focus of "pathogen free" is at the point of consumption? Meaning then that the meat leaving the processing plant must also be pathogen free. Which also implies that the conditions of distribution, storage and handling would also be effective to preclude contamination. Each of these steps have very unique challenges. My first instinct was to look at the meat animals to see if they could somehow be produced absent the pathogens. That would seem to be an exceedingly costly undertaking and then it would be very unlikely that the pathogen-free animals could be further processed without the possibility of recontamination. So, then I think that an intervention technology at the terminus of the

processing line and likely post packaging would be required to achieve the pathogen-free state. Ionizing radiation and perhaps HPP might be good candidates for this treatment step. PEF might also work. But we know that consumers remain reluctant about the use of radiation so that one would likely fall out of favor. HPP and PEF are worth a look. They are both very well received by regulatory agencies, industry experts, and consumer groups. Proving these technologies will require some investment. I've seen PEF machine down in Australia that I think is capable of doing this job. The PEF machine is located in the CSIRO lab in Werribee (Melbourne), but you don't have to go that far to access the device or to learn more about PEF. Stefan Toepfl over in Germany is leading the charge at the moment in the advancement of PEF technology. I believe that he could add depth and granularity to the discussion of the technology. The question that I can't answer at the moment is about contamination occurring following the PEF application and treatment.

Input fra Roy Betts | Microbiology Ambassador | Campden BRI | Chipping Campden, UK

On meat, in UK the general feeling is committed meats are eaten fully cooked, although some high end restaurants can give an option of rare burgers (there is much control over this). The UK FSA, give an option of rare cooked whole muscle cuts of beef and lamb, but not pork (due to trichinella and Hep E concern). Poultry is always to be fully cooked. But we do get issues. The main meat related food poisoning issue is from campylobacter in poultry, biggest cause of food poisoning cases in UK. We had a big issue recently with chicken nuggets causing salmonellosis. Cause: flash fried nuggets being warmed up rather than cooked properly, as instructions tell to do. Overall if you could get pathogen free chicken, UK may have around 20 to 40 thousand less cases of poisoning each year.

The main bacterial pathogen I'd link with fresh pork is *Salmonella*. *Listeria* wouldn't really be considered, and generally STEC is low prevalence. However, if the requirement is no pathogens, I guess we may be thinking no bacteria at all. Of course, the work "no" needs definition. We in Europe don't tend to believe zero is possible, so some objective for no pathogen or pathogen reduction is needed. However, I do think that people thinking no pathogens should not just restrict to bacterial pathogens, in pork trichinella and Hep E should be considered, particularly as Hep E may have higher heat resistance than bacterial pathogens.

Input fra Dr. Roger Cook, PhD | Konstitueret direktør for fødevarevidenskab og risikovurdering | Direktoratet for Fødevarevidenskab og Risikovurdering | New Zealand Food Safety

What we are talking about is really reducing the microbial load on meat to the extent possible that there is negligible risk to human health if the food is handled and prepared properly in the kitchen. In effect, there aren't too many pathogens to spread around the kitchen before the meat is cooked, the cooking process eliminates or reduces the level of pathogens to below the infectious dose (Just Cook It), and the cooling and storage processes prevent germination, growth and toxin production by spore-formers. Easy as that.

So primary processors have to implement GHP (Good Hygiene Practices) to the best extent possible to meet the above goal as well as not add other risks, for example proven chemical or socio-economic/political risk. Easy to produce pathogen free meat but the cost to the consumer would be horrific and politicians would shake in their boots.

With respect to NZ, we identified *Salmonella*, *Yersinia*, *Trichinella*, *Toxoplasma* and *Hepatitis* E as pathogens of interest but none of them have been of strong concern here in New Zealand as our farm husbandry practices and processing standards have, as noted above, minimised their presence. We had specific measures in place around tonsils to mitigate the risk of *Yersinia* and are in the process of reviewing them in the face of an increase in notified human cases over the last year. While it is possible that processing standards have slipped, it is equally plausible that the huge interest now in Asian pork dumplings that can be bought frozen, and hence undercooked, might be the reason for the increased notifications.

You ask about levels. If we consider all biological populations to follow a lognormal count distribution curve, then as we reduce the numbers by moving the curve to the left, the prevalence will drop given most enumeration tests have a lower limit of detection. And of course, there will be some level of elimination. So yes, exposure is not just reduction in prevalence but also dropping the high counts. The two are interrelated.

To answer your question "is the 'pathogen-free meat' trend is something you see in your culture/corner of the world as well?": No – our manufacturers and consumers understand that pathogens are generally normal flora of animals or the environment, but they do expect upstream companies to do their best, within reason, to minimize contamination. And then of course we have *Campylobacter* on chicken meat. Just Cook IT!! Then again – irradiate!!!!!!

Input fra Julian Cox | Honorary Associate Professor | School of Chemical Engineering | UNSW, AUS

When addressing the questions regarding pathogen free poultry, one has to look at the issues and risks: Regarding prevalence, we have to consider the rate of contamination and to do an exposure assessment. After that, it is necessary to look at the level of contamination and then further refine the exposure assessment accordingly. We need to look at population(s) of what. There is *Salmonella* and then there is *SALMONELLA*, which is exemplified with the experiences with *S. Enteritidis* versus *S. Sofia*:

Salmonella Enteritidis	*Salmonella Sofia*
Colonises chickens very well (extraintestinal)	Colonises chickens very well
Highly virulent in chickens (morbidity, mortality)	No disease in chickens
Illness in humans (egg-associated)	No illness in humans
Major public health concern	No public health concern

To ensure food safety, one has to manage/intervene at all steps: primary production (the farm), processing (the 'factory'), distribution (wholesale, retail), and consumption (the home). In primary production, ways to address are breeding, hygiene management at hatchery, feeding (competitive exclusion), vaccination, immunotherapy, and phage therapy. During processing, choosing the best equipment (e.g., eviscerator, inside-outside washer, and spin chiller) is paramount as well as having a good HACCP plan, solid risk assessments, and a sensible testing plan using validated analytical methods.

9. Diskussion

De teknologier, der er beskrevet i afsnit 6, har alle potentiale for anvendelse på brystflæsk. Alle teknologierne har deres styrker og svagheder, men ingen af metoderne anvendt alene kan forventes at resultere i mere end en mikrobiel reduktion på max. 2 log (tabel 7), uden at gå på kompromis med andre kvalitetsparametre eller at skulle løse nogle meget svære problemstillinger (fx afskaffelse af radioaktivt affald og negative forbrugerholdninger, som det er tilfældet med gamma bestråling).

Tabel 7. Dokumenteret effekt på fersk kød for hver af de gennemgåede metoder/teknologier

	Listeria reduktion	*Salmonella* reduktion	Holdbarhedsforlængelse
Bakteriofager	1-2,3 log	1-4 log	4 dage[6]
eBeam	-	2-5 log	14 dage[7]
Gamma	1,5-5 log	-	>20 dage[8]
Røntgen (X-ray)	-	-	-
HPP/UHP/HHP	>5 log[9]	>5 log[9]	6 dage[10]
Klor	1-3 log	1-3 log	-
Kold plasma	>1 log	>1 log	-
Ozon	1-6 log	>1 log	2-6 dage
PEF	-	-	-
Ultralyd	1 log	1-2 log	-
UV-C	-	0,4-3 log	-

I tabel 7 er de dokumenterede effekter for hver teknologi mht reduktion af *Listeria* hhv. *Salmonella* samt holdbarhedsforlængelse, samlet. Når man læser tabellen, er det vigtigt at huske:

[6] *Brochothrix*-specifik bakteriofag anvendt på fedtvæv fra gris. Holdbarhedsforlængelse fra 4 til 8 dage (Kazi & Annapure 2016)
[7] García-Márquez et al. 2012
[8] 3 kGy/0°C (Bari et al. 2006), kombineret med MAP (Pillai & Shayanfar 2017)
[9] Kombineret med carvacrol (bilag 5, tabel 2)
[10] Ved 4°C

- Der kan være dokumenterede studier, der ikke er fanget i søgningerne
- Effekter opnået ved eBeam, gamma eller røntgen kan til en vis grad samles i effekter ved bestråling, idet teknologierne adskiller sig på strålekilden, dosishastigheden og gennemtrængningsdybden, men at selve påvirkningen af bakteriecellerne (eller sporerne) er sammenlignelig
- Det at der ikke er dokumenteret effekt på fersk kød, betyder ikke at teknologien af den grund skal dømmes ude – alle teknologierne er forholdsvis nye og de studier der er foretaget indtil videre, tager udspring i de mest presserende problemstillinger, fx *Listeria* i RTE-produkter (dvs. ikke fersk kød).

De teknologier der skiller sig ud, er umiddelbart bestråling, HPP og ozon, men dykker man lidt ned i dem, vil man hurtigt finde nogle forhold, der trækker lidt ned i regnskabet: for gamma bestråling er der det radioaktive affald og logistik- og kapacitetsproblemer (eneste sted i DK der kan gamma-bestråle er Risø); resultaterne i tabellen for HPP ser fornuftige ud, men er opnået ved at kombinere med et planteekstrakt; den meget flotte *Listeria* effekt af ozon er set ved forsøg hvor der er anvendt meget høje koncentrationer af ozon i gasform (som teknologisk og arbejdsmiljømæssigt er problematisk) og forsøget tager ikke stilling til eventuelle uønskede indvirkninger på ikke-mikrobielle kvalitetsparametre. Ikke overraskende er der ingen løsning der er attraktiv på alle parametre. Men det er værd at bemærke, at flere undersøgelser har vist, at kombination af ikke-termiske metoder med andre ikke-termiske metoder eller med konventionelle metoder kan have en gavnlig hurdle-effekt, der kan påvirke den mikrobielle belastning med en additiv eller endda synergistisk virkning, samtidig med at den har mindre konsekvenser for de sensoriske og ernæringsmæssige parametre (Sethi et al. 2019 og bilag 8). Derfor skal en løsning måske søges i en kombinationsbehandling.

Integritet
En vigtig del af overvejelsen er integriteten af produktet efter dekontamineringen, dvs. hvor sikker kan man være på at der ikke sker en efterkontaminering af produktet. Dette kan sikres ved enten at dekontaminere umiddelbart inden produktet emballeres eller når den allerede er emballeret. Skal behandlingen foregå umiddelbart inden pakning skal teknologien kunne integreres med en pakkemaskine, som fx den der er vist i figur 12. Aktuelle metoder vil være bakteriofager, klor, ozon, PEF og ultralyd. Skal behandlingen foregå efter emballering, kan man vælge at inkorporere teknologien umiddelbart efter pakkeprocessen, som fx vist i figur 13. HPP, kold plasma og UV-C teknologierne vil være relevante i denne sammenhæng og muligvis også røntgen bestråling. Derimod vil eBeam bestråling kræve så omfattende udstyr og sikkerhedsforanstaltninger, at der må påregnes at skulle opføres en særskilt bygning til dette formål. Gamma bestråling kræver en særlig myndighedstilladelse, da der skal håndteres radioaktivt materiale, der udgør en samfundssikkerhedsmæssig risiko. Så vidt vides er det i Danmark kun Forskningscenter Risø, der har tilladelse til at udføre gamma-bestråling.

Figur 12. Mulig placering af dekontamineringsteknologi, der skal udføres inden emballering.

Figur 13. Mulig placering af dekontamineringsteknologi, der skal udføres efter emballering.

Omkostning

At håndtere naturlig variation i råvaren er en klassisk og uundgåelig udfordring i fødevareindustrien. Lave mikrobielle belastninger er ønskelige, da lave kimtal generelt vil resultere i en længere holdbarhed og indikere et sikrere produkt. Spørgsmålet er, om værdien af dekontaminering vil overstige omkostningerne ved at gennemføre en dekontamineringsprocedure. Alle de beskrevne dekontamineringsmetoder i denne rapport vil som sagt have en meget beskeden indvirkning på mikrobielle belastninger uden at gå på kompromis med andre kvalitetsparametre. Det skal derfor overvejes nøje, hvor stor værdi f.eks. en reduktion på 0,5, 1,0 eller 2,0 log ville udgøre for industrien. Baseret på prædiktive modeller (FSSP og Combase) vil en reduktion på 1 log resultere i en mikrobiel holdbarhedsforlængelse på 1-2 dage. Hvis en kombination af metoder kunne skabe en synergistisk effekt og måske give en 3-log reduktion af den mikrobielle belastning, ville det teoretisk give 3-6 dages ekstra holdbarhed til produktet. Vil det retfærdiggøre investerings- og driftsomkostningerne ved dekontaminering?

Lovgivning

Det var hensigten at kaste lys over den lovgivningsmæssige status for hver enkelt teknologi, men denne hensigt er blevet alvorligt udfordret af manglen på tilgængelige oplysninger, den vage information i de oplysninger, der kunne findes, og den generelle usikkerhed om især EU's lovgivende organers holdning. Et kompendium af "Potential Fish Decontamination Treatments" (https://www.foodstandards.gov.scot/self-assessment-resources/potential-fish-decontamination-treatments) udarbejdet af Food Standards Scotland (FSS) illustrerer dette perfekt. Selvom kompendiet fokuserer på reduktion af *Listeria* i røget laks (snarere end fersk kød), illustrerer det rigtig godt, hvor forvirrende de tilgængelige oplysninger er. Fx står der: 'En hel del forskning, der spænder over flere årtier finansieret af WHO, FN-FAO og USDA, har vist, at bestråling af fødevarer generelt er sikker og en effektiv måde at dræbe bakterier på og konservere mad', men i EU skal bestrålingen af fødevarer godkendes af Europa-Kommissionen fra sag til sag, og fisk og skaldyr kan udsættes for op til 3 kGy, forudsat at der er et rimeligt teknologisk behov (...). Så på den ene side har tre velrenommerede organisationer/myndigheder (WHO, UN-FAO og USDA) i det væsentlige givet bestråling et godkendelsesstempel, men alligevel sætter EU vejspærringer op for industrien, hvilket effektivt forhindrer implementering, da bevisbyrden lægges på fødevareproducentens skuldre. En lignende situation foreligger for behandling med High Hydrostatic Pressure (HHP/HPP), hvor forfatterne til FSS-kompendiet bemærker, at 'situationen for anvendelse af HPP er mindre end klar i EU. Der har været drøftelser i EU om lovligheden af at bruge HPP, men de seneste konsolideringer af dokumenter (fx EF 852/2004) nævner ikke specifikt HHP'. Et tredje eksempel er UV-C-dekontaminering af fødevarer, hvor USDA regulerer UV-C-behandling som tilsætningsstof, mens 'UV-behandlede fødevarer tilhører kategorien Novel Foods i EU, Storbritannien, Canada, Australien, New Zealand og Kina. Novel Foods og ingredienser reguleres forskelligt i hvert land, hvor de fleste systemer er baseret på en risiko- eller fødevaresikkerhedsvurderingsmodel, og hvor de fleste lande desuden kræver ansøgning og godkendelse (...) De vigtigste hindringer for kommercialisering [af UV-teknologien] relateret til investeringsomkostninger, fuld kontrol over variable forbundet med procesoperationen og manglende lovgivningsmæssige godkendelser og retningslinjer, har forsinket en bredere og hurtigere implementering af UV-teknologien i industriel skala' (Koutchma 2018). For at få en dybere forståelse af betingelserne omkring EU-

lovgivningen for de teknologier og metoder, der behandles i denne redegørelse, blev der søgt rådgivning hos prof. dr. Bernd van der Meulen, en fødevarejuridisk konsulent ved og direktør for firmaet 'European Institute for Food Law'. Bernd van der Meulen's analyse er inkluderet i afsnit 11 i denne redegørelse. EU-lovgivningen skelner mellem kemiske stoffer (substances), mikroorganismer og processer. Bern van der Meulen har valgt at slå kemiske stoffer og mikroorganismer sammen til en kategori han kalder 'Agents' (midler). Af de metoder der er gennemgået i denne redegørelse, vil midlerne være bakteriofager, klor og ozon – resten betragtes som processer. Hvad angår midlerne, vurderer van der Meulen at klor kan anvendes i en koncentration på højst 250 µ/liter, at bakteriofager kan anvendes som hjælpestof, dvs. hvis de ikke har en funktion i det endelige produkt, men ozon vil kræve en forudgående godkendelse. Hvad angår processerne, vurderer van der Meulen at UV kan bruges til dekontaminering uden forudgående godkendelse og det samme gælder for kold plasma, pulserende lys og eBeam, dog med det forbehold at det skal kunne vises at der ikke opnås en signifikant bedre effekt end hvis konventionelle metoder var anvendt. I modsat fald, vil det muligvis være nødvendigt at søge om tilladelse til brug under 'Novel Food' lovgivningen.

Denne rapport skal fungere som et redskab for kødindustrien, til at indsnævre dekontamineringsteknologier, der berettiger til et nærmere kig, dvs. ville være værd at undersøge i en række eksperimenter udført af DMRI i 2023. Afvejning af fordele og ulemper og beslutning om en vej fremad vil omfatte at træffe nogle hovedbeslutninger baseret på økonomiske begrænsninger, etiske værdier, presserende behov/værdi for kødindustrien og kundernes forventninger/krav.

10. Litteraturoversigt

En litteraturoversigt findes i et separat Excel-dokument, hvor alle videnskabelige artikler og andre artikler er opført med forfatter/titel, formål samt en kort beskrivelse af væsentlige resultater/konklusioner. Dokumentet er beregnet til at blive brugt interaktivt, da posterne kan sorteres efter fx udgivelsesår, forfatter eller teknologitype.

11. Analyse af lovgivningsmæssige aspekter

Decontamination in EU food law

Explorative report

by Bernd van der Meulen[11]
Date 2 december 2022

Abstract

Many uncertainties surround the regulatory situation of food decontamination in the EU. For this reason, the current analysis can only conclude in terms of probabilities. Whether the conclusions will stand depends on the willingness of authorities and ultimately the courts to accept the reasoning presented here.

A general prerequisite of decontamination is that the resulting food is safe and fit for consumption.

With regard to the use of substances for the purpose of decontamination, this analysis concludes that Nisin E 234 can only be used as processing aid i.e. if it does not have a function in the final product, chlorine can be used in a concentration of 250 microgram/litre, ozone and PAA would require prior authorisation.

For all substances covered in this project an application for authorisation as decontaminant is worth considering.

With regard to bacteria, the analysis concludes that those lacto acid bacteria that are on the QPS list as well as lactobacillus reuteri can be used for the purpose of decontamination. Bacteriophages can be used as processing aid i.e. if they do not have a function in the final product.

With regard to the use of processes for the purpose of decontamination, this analysis concludes that UV can be freely used for the purpose of surface decontamination.

Cold plasma, pulsed light and eBeam can be freely used in so far as the level of decontamination they achieve is not significantly different from what is achieved with conventional methods of decontamination. If it is considerably different, prior authorisation under the Novel Foods Regulation may be required.

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Introduction

Objective

The objective of the DTI overall project is to map out decontamination solutions relevant for the fish and meat industry respectively with the purpose of improving and homogenising the overall microbial quality of the finished products. In my interpretation the concept 'decontamination' as used in these studies includes preservation. The objective of this analysis is to gain understanding of the food legal situation and the regulatory challenges in the EU.

Concern

As rightfully stated in the DTI guideline the intention to bring to light the legislative status of each technology has been severely challenged by lack of available information. Decontamination is a little explored field in EU food law and many uncertainties exist. For this reason, the level of clarity that I can provide in this report is limited. At several points I have no more to offer than interpretations that are 'defendable'. Whether authorities will actually accept these interpretations cannot be guaranteed but hopefully they will be open to discuss this with you. What I can do is provide a structure and classifications that may help to ask the right questions and find the applicable regulatory framework.

Background

Key objective of EU food law are to protect human life and health and other consumer interests (Article 5 of Regulation (EU) 178/2002). In this regard it is prohibited to place unsafe foods on the market (Article 14 of Regulation (EU) 178/2002) and it is mandatory for food businesses to follow good hygiene practices (Regulation (EC) 852/2004 on food hygiene and Regulation (EC) 853/2004 laying down specific hygiene rules for food of animal origin). The methods discussed in the DTI guideline may help in implementing hygiene.

Generally the EU is reluctant towards decontamination. Emphasis is on prevention. It seems to be feared that decontamination may facilitate sloppy prevention practices.

Agents and processes

The DTI guideline covers the use of chemical substances, microorganisms and processes for the purpose of counteracting pathogenic microorganisms. As will be set out below, the regulatory framework that applies to substances and organisms on the one hand (hereinafter together referred to as 'agents') and to processes on the other hand may be different. For this reason, I will discuss separately the agents and the processes.

Agents

Introduction

Decontamination probably is a technological function within the meaning of Regulation (EC) 1333/2008 on food additives. It is covered by the functional class of preservatives. By consequence, agents used for decontamination purposes may classify as food additives.

Additives may be used in accordance with the provisions in the additives regulation. Agents that do not have a function in the final product escape from the applicability of the additives regulation. They are so-called 'processing aids'. Processing aids fall both outside the authorisation requirement and outside the labelling requirement for additives (Article 2(2)(a) Regulation (EC) 1333/2008).

For processing aids separate legislation may exist. We find authorisation requirements in some EU member states including, France, Spain and the Netherlands. This is not further discussed here. At EU level processing aids used for decontamination are within the scope of Article 3(3) of Regulation (EC) 853/2004 as discussed below.

To distinguish processing aids and additives used for decontamination, it is important to assess whether the agent has a function in the final product. I.e. does the decontamination still continue or does it stop at some point during processing?

Additives

Substances[12] that are not normally consumed as a food and are added for a technological purpose are food additives. Kitchen salt for example is normally consumed as a food and therefore is not a food additive if it is used for the purpose of decontamination.

Food additives may only be used if they have been authorised and only in the food categories for which they are authorised. This is indicated in the annexes to the additives regulation.

For the current project several food categories in these annexes may be of relevance. These are the categories 08.1 fresh meat, 08.2 meat preparations, 09.1 unprocessed fish and fisheries products, 09.1.1 unprocessed fish and 9.2.2 Unprocessed molluscs and crustaceans.

For most categories of food products E 290 carbon dioxide and E 948 oxygen are authorised to be used as preservatives.

[12] As the additives regulation applies to 'substances', it can it be argued that (almost) living organisms such as bacteria and viruses are not 'substances' and therefore are outside the scope of the additives regulation. In the current context it is not possible to further analyse this thought.

For fresh meat, no preservatives have been authorised. For a very limited number of meat preparations the following preservatives are allowed: E 220-228 Sulphur dioxide — sulphites; E 249-250 Nitrites; E 260 Acetic acid, E 261 Potassium acetates, E 262 Sodium acetates, E 263 Calcium acetate, and E 270 Lactic acid. On unprocessed fish among others E 300 Ascorbic acid and E 330 Citric acid may be used, but no specific preservatives. On certain unprocessed molluscs and crustaceans E 220-228 Sulphur dioxide — sulphites may be used.

At present these substances are not covered by the DTI project. I mention them here as they have already passed safety assessment. It may be worth considering to apply for expansion of the scope of application of existing food additives if they might be useful or for the authorisation of additional additives. Moreover, in so far as these substances do not have a function in the final product, they may be used as processing aids also in foods for which their use as additives has not been approved, provided this use is safe for consumers.

Nisin E 234
Nisin is on the list of authorised food additives. So far its use has only been authorised in certain dairy and egg products. As Nisin has already successfully passed an additives safety assessment, an application for expansion of its scope of application may be worth considering.

Processing aids
Agents that have no function in the final product escape the strict requirements for food additives. If no specific legislation exists, they may be freely used as long as they are safe.

However, to different types of products, some different regimes apply as regards their decontamination. According to Article 3(2) of Regulation (EC) 853/2004 for the removal of surface contamination from products of animal origin only potable water may be used as well as substances authorised for this purpose.

> Food business operators shall not use any substance other than potable water or, when Regulation (EC) No 852/2004 or this Regulation permits its use, clean water, to remove surface contamination from products of animal origin, unless use of the substance has been approved by the Commission. For that purpose the Commission is empowered to adopt delegated acts in accordance with Article 11a supplementing this Regulation. Food business operators shall also comply with any conditions for use that may be adopted under the same procedure. The use of an approved substance shall not affect the food business operator's duty to comply with the requirements of this Regulation.

Based on the text of this provision, decontamination that does not aim to remove contamination from the surface but from the product internally ('through and through') is not covered. For this reason I will

distinguish surface decontamination and full decontamination.[13] The substances covered in this project are all considered for the purpose of surface decontamination. Therefore they are all potentially within the scope of this provision.

Chlorine
Chlorine is used for the decontamination of surfaces. Therefore, it is covered by Article 3(2) of Regulation (EC) 853/2004. Chlorine has not been authorised by the European Commission for this purpose. However, in practice, chlorinated water is often used for the purpose of decontamination. This may be compatible with Article 3(2) of Regulation (EC) 853/2004 in so far as drinking water may be chlorinated. According to Belgian authorities, the maximum level for free chlorine residues in drinking water is 250 microgram/litre.[14]

Position on chlorine
Within the limits that drinking water may be chlorinated, it is allowed to use chlorinated water for decontamination of surfaces of products of animal origin.

Ozone
Ozone is used for the decontamination of drinking water. Apparently, it is considered a food grade technology.

As indicated above, oxygen is authorised as food additive and may be used in most foods. Unfortunately in this context oxygen is defined as O_2 (Regulation (EC) 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008) and therefore does not include ozone (O_3).

Position on ozone
At present, due to Article 3(2) of Regulation (EC) 853/2004 does not seem to be allowed for use in surface decontamination of foods of animal origin. Given its favourable safety profile, an application for authorisation seems promising.

[13] Moreover, Article 3(2) of Regulation (EC) 853/2004 only applies to products of animal origin. At EU level, no such provision applies to products of plant origin. By consequence, for the purpose of decontamination during processing of plant based products processing aids may be used if they do not have any function (effect) in the final product, except when other legislation states otherwise. For organic products, for example, only processing aids may be used that are included in a list of authorised substances. Given the context of the current study, I will limit myself here to products of animal origin.

[14] https://water-link.be/tips-advies/kwaliteit-en-samenstelling/chloor#:~:text=In%201%20liter%20drinkwater%20mag,50%20microgram%20aan%20vrije%20chloorresten. A source from the Netherlands gives the same limit: https://www.google.nl/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwjpx9f0y8n7AhVS26QKHTFFC2sQFnoECC0QAQ&url=https%3A%2F%2Fwww.helpdeskwater.nl%2Fpublish%2Fpages%2F165197%2Fhandleiding_chloridenormering_drinkwaterbronnen_helpdesk_water.pdf&usg=AOvVaw3G8iZNq3BzjJW4w7nfGB8K.

Peracetic acid (PAA)
Also peracetic acid (PAA) only plays a role on the surface and therefore is fully within the scope of Article 3(2) of Regulation (EC) 853/2004. According to the DTI guideline EFSA already found it safe. As far as I know no application for authorisation has been submitted in the EU. PAA is known to decompose quickly into harmless substances. Given its favourable safety profile, an application for authorisation seems promising.

Position on peracetic acid (PAA)
It seems recommendable to apply for authorisation of PAA for use as decontaminant in the EU.

Biopreservation
The DTI guideline addresses biopreservation through lactic acid bacteria, fermentation with *Lactobacilis reuteri* and through bacteriophages.

QPS
In the course of its work, EFSA has been confronted in different contexts with questions regarding the safety of the use of micro-organisms in food processing and human consumption. EFSA has collected the findings of its assessments in so far as they are favourable in a database known as the QPS-list. QPS stands for 'qualified presumption of safety'. The QPS-list has no formal regulatory status. However, it does provide an indication that the organisms that are listed are not novel or have been authorised. In practice authorities consider the use of QPS listed organisms a matter of low concern. By consequence, the regulatory risk of using QPS organisms seems to be limited.

Limosilactobacillus reuteri commonly known as *Lactobacillus reuteri* is included in the QPS list. The DTI guideline does not further specify the lactic acid bacteria, but several of these are included in the QPS list. Phages are not included. Phages are discussed below.

Position on biopreservation
The use of lactic acid bacteria that are included in the QPS list and the use of fermentation through *Lactobacillus reuteri* can be considered acceptable from a regulatory point of view.

Phages
Phages are possibly intended to be used for the decontamination of a product 'through and through'. For this reason I would argue that they may be outside the scope of Article 3(2) of Regulation (EC) 853/2004 and may be used freely if they qualify as processing aids and require authorisation if they qualify as food additives.

Within the EU phages have been addressed in the context of a product (Listex™) designed to combat *Listeria*. With regard to the application on cheeses, the European Commission has asked EFSA for an interpretation whether they have a function in the final product. According to EFSA viruses present in soft cheeses can combat a new infection with *Listeria* and therefore they have a function in the final

product. In hard cheeses they are unable to reach a new infection and they have no function. As far as I know, no further conclusion has been drawn. However, I understand this to mean that according to the European Commission phages are food additives in soft cheeses and therefore may not be used except when authorised. In hard cheeses they are processing aids and can freely be used as long as they are safe.

Position on phages
It must be assessed whether the phages have a function in the final product. If they do not, they may be freely used. If they do, it is recommended to submit an application for authorisation as food additive.

Process

Introduction
Article 3(2) of Regulation (EC) 853/2004 does not apply to processes that do not involve agents. Such processes must be assessed on their own merits. To irradiation a specific legal framework applies. Processes to which no specific framework applies may come under the Novel Foods Regulation (NFR). See below.

Irradiation
Framework
In the EU many consumers seem to be opposed to the use of irradiation which they perceive as an application of nuclear power. By consequence the legal framework can hardly be seen as enabling and options to use irradiation for food safety have been explored only to a limited extent.

Legislation in the EU Member States is harmonised through Directive 1999/2/EC on foods and food ingredients treated with ionising radiation and Directive 1999/3/EC on the establishment of a Community list of foods and food ingredients treated with ionising radiation. It is only allowed to place irradiated foods on the market in EU if they comply with the legislation. (For an overview of the framework on irradiation beyond the EU see GHI 2018.[15])

Harmonisation of authorisations has not been successful. Authorisations differ considerably among Member States. As far as relevant in current context, in Belgium and the Czech Republic (and the UK) irradiation is allowed for poultry, fish and shellfish; in France for poultry, in the Netherlands for chicken

[15] In particular the document provides information on foods allowed to be irradiated in countries such as the USA, Canada, the EU, Bangladesh, China, India, Indonesia, Malaysia, Pakistan, Japan, the Philippines, Thailand and Vietnam. It concludes that it is clear from the literature and widespread safe use that ionizing radiation is a beneficial and necessary technology for control of food infestation and prevention of foodborne pathogens. Legislation should not impede technological innovations that can provide solutions for food safety and security, while also contributing to sustainability of food chain, reducing consumption of resources (e.g. energy) and generation of waste.

meat and shrimps. In other EU Member States irradiation of meat and fish is not allowed. For all practical purposes, the methods covered in this project can be used only in so far as they are not covered by the legislation on irradiation.

Scope
The legislation applies to products treated with ionising radiation. However, the Directive does not define the concept ionisation. In the current context we need to explore whether eBeam, UV, cold plasma and pulsed light are or are not covered by this concept. If they are, to a large extent their application must be considered prohibited in the EU. If they are not, they must be assessed under the frameworks addressed in later sections.

According to Annex II of Directive 1999/2/EC "Foodstuffs may be treated only by the following sources of ionising radiation: (a) gamma rays from radionuclides ^{60}Co or ^{137}Cs; (b) X-rays generated from machine sources operated at or below a nominal energy (maximum quantum energy) level of 5 MeV;(c) electrons generated from machine sources operated at or below a nominal energy (maximum quantum energy) level of 10 MeV."

It is not entirely clear whether the directive considers other sources of radiation to be outside the scope of the legislation on irradiation, or considers them to be prohibited by legal definition. National implementing legislation may shed some light on this issue.

In the Netherlands, the Directives on irradiation are implemented by the Warenwetbesluit Doorstraalde waren (Commodities Act Decree on irradiated commodities). This decree prohibits the treatment of food products with ionising rays except when this treatment complies with the provisions of said Decree. The scope of application of the Decree hinges on the concept 'ionising rays' and 'ionising radiation' respectively. For the definition of ionising rays, the Decree refers to the Kernenergiewet (Nuclear Energy Act). This act defines ionising radiation[16] as: Röntgen (X-ray) and gamma rays and particulate radiation, capable of causing the formation of ions.

'Particulate' of 'corpuscular' radiation is radiation consisting of atomic or subatomic particles (such as electrons). The concept is used as opposed to electromagnetic radiation (such as microwave, visible light and UV).

In the UK the same interpretation is found. According to Article 3 of The Food Irradiation (England) Regulations 2009 "ionising radiation" means any gamma rays, X rays or corpuscular radiations which are capable of producing ions either directly or indirectly.

[16] Apparently, the concepts 'ionising rays' and 'ionising radiation' are used interchangeably.

From these definitions would follow that UV, for example, does not constitute ionising radiation under English or Dutch legislation: it is not X-ray, nor gamma, nor particulate/corpuscular. This is regardless if in fact it does or does not produce some ionisation.

The German Decree on Food Irradiation[17] states in its text that the treatment of herbs and spices with the three sources of radiation is allowed. In its annex, it states that foodstuffs may only be treated with these sources of radiation. The Decree further states that the treatment with UV is allowed of drinking water, surfaces of fruit and vegetable products and hard cheese. Further indirect application to food via UV treatment of air is allowed. Apparently, the German legislature is of the opinion that treatment with UV is not covered by the prohibition (with authorisation exception) in the Directive.

In case of discrepancies, national legislation should be interpreted in conformity with the European directive. The Directive covers gamma rays, X-rays, and electrons. However, it is less clear whether other rays are considered non-ionising by legal definition as in the English and Dutch legislation and thus outside the scope of the legislation, or if they are excluded from use in case they would in fact be ionising. As stated above, the Directive uses the expression 'ionising radiation' but does not provide a definition. This may mean that recourse must be taken to common use of this expression. Common use of the expression 'ionising radiation' would take place in the domain of physics. However, it is not easy to derive a clear delineation from physics. In literature, UV to stay with the example, is generally classified as non-ionising (Motarjemi and Lelieveld 2014, p. 230). However, it has been pointed out that the shortest ultraviolet wavelengths do bring about some ionisation (Shama 2007). UV forms part of the electromagnetic spectrum and the UV wavelength range is from about 10 to 400 nm, placing it between X-rays and the visible part of the spectrum (Shama 2007). The wavelength ranges that may produce ionisation reaches up to about 100 nm (University of Twente 2018). But even this limit is not razor sharp. In fact, all radiation, including sunlight, is known to bring about some ionisation. It is obvious that the legislator cannot have intended to bring sundried products (such as tomatoes in Italy or Greece) within the ambit of the legislation on irradiation. To achieve the result that such products are in fact outside the scope of the legislation, some limitation to the concept of ionisation is needed.

The solution chosen by the English and Dutch legislature is attractive in that it is clear cut. On this basis I take the position that radiation other than X-ray, gamma rays and electrons is outside the scope of the legislation. For this reason I consider defendable that UV, cold plasma and pulsed light as covered by the project is outside the scope of the legislation on irradiation.

[17] Verordnung über die Behandlung von Lebensmitteln mit Elektronen-, Gamma- und Röntgenstrahlen, Neutronen oder ultravioletten Strahlen (Lebensmittelbestrahlungsverordnung - LMBestrV) < https://www.gesetze-im-internet.de/lmbestrv_2000/BJNR173000000.html >.

UV

Based on the general theory on irradiation set out above, I would argue that UV treatment is outside the scope of the legislation on irradiation.[18]

In the DTI guideline, reference is mad to UV treated products that are within the scope of the Novel Foods Regulation (as set out below). However, that precedent is about the treatment of mushrooms with UV with the purpose of increasing the production of vitamin D, not with the purpose of decontamination. In my view, this precedent does not apply in our context.

Position on UV

In my view a reasonable interpretation would place UV treatment outside the scope of the legislation on irradiation. Also, UV treatment has a history of safe use in the EU prior to 1997 and for this reason is outside the scope of the Novel Foods Regulation. Therefore, I would argue that the use of UV for decontamination purposes is allowed in the EU without authorisation requirement.

Cold plasma

Cold plasma is a novel non-thermal food processing technology that uses energetic, reactive gases to inactivate contaminating microbes on the surface of foods. The gases are made ionizing with corona discharges, as applied in ozone generators (i.e. electricity).

Position on cold plasma

For the same reasons as set out above, I take the position that cold plasma is outside the scope of the legislation on irradiation. As cold plasma to the best of my knowledge has not been applied in the EU prior to 1997, it may need to be assessed under the Novel Foods Regulation. See below.

Pulsed light

Based on the general theory on irradiation set out above, I would argue that treatment with pulsed light is outside the scope of the legislation on irradiation. As pulsed light to the best of my knowledge has not been applied in the EU prior to 1997, it may need to be assessed under the Novel Foods Regulation. See below.

eBeam

The situation seems more complex with regard to eBeam. eBeam is a novel technology using low energy electrons to decontaminate product surfaces preserving, for example, germination potential and nutritional value of foods. It seems to be covered by the category "electrons generated from machine sources operated at or below a nominal energy level of 10 MeV" and therefore is potentially within the scope of the legislation on irradiation.

[18] This also seems to follow, for example from Article 25s of Commission Regulation (EC) 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control.

The legislation on irradiation addresses a treatment of the food 'through-and-through'. eBeam by contrast is designed for surface decontamination. It does not affect the inside of the product. Similar to the discussion above, the consequence has to be one of two extremes. Either it means that eBeam is outside the scope of the legislation, or the application of eBeam for the purpose of surface decontamination is prohibited.

In different language versions of the Directive, words are used either expressing that the radiation goes through the product (Dutch "doorstraling" the element "door" equals "through") or that the radiation goes on the product (German "Bestrahlung" the element "be" equals "on"). In connection with the dosimetry discussed hereafter, technologies such as surface treatment may either be outside the scope of the legislation (if the scope is on radiation that goes through the product) or inside the scope (and probably prohibited if it also applies to non-penetrating radiation).

As surface treatment did not exist at the time the Directive was drafted, it may not be entirely a language issue. Urban, for example, wrote in 1986: "one necessary property of ionizing radiation used in food irradiation is its ability to penetrate food". It seems probable that, regardless of the exact words chosen, what the legislature had in mind was radiation affecting the food "through and through".

Annex III of Directive 1999/2/EC elaborates the concept dosimetry. Section 1 ends by stating: "The ratio of Dmax/Dmin should not exceed 3." This means that the lowest absorbed dose in the treated product should not be more than 3 times lower than the highest absorbed dose. In phrasing this requirement, the legislature must have had the situation in mind where a product is radiated "through and through". If there are parts within the product where a sufficiently high dose is not reached, these parts may constitute safe havens for pathogens.[19]

This dosimetry requirement creates a challenge for surface treatment. In case "only" the surface of the product is treated with low energy, the absorbed dose inside the product will be zero. If the Dmax is divided by zero, the outcome is infinite (which is by far higher than 3). By consequence, if surface treatment is within the scope of the legislation, it is not allowed to be applied under any circumstance. Except, if one could argue that the Dmin should not be measured anywhere in the product, but that the business operators enjoy discretion in deciding which parts of a product they do wish to treat, and which parts not. In such reading of the law, in case of surface treatment the Dmin should be measured somewhere at the surface.

The provision on dosimetry, however, does point in the direction that the legislature was thinking of radiation "through and through". Also, the high energy sources of legislation included in the legislation point in this direction. From the perspective of protecting consumers from the risks of radio activity, there does not seem to be any need to include surface treatment in the scope of the legislation.

[19] This is also the reading followed by EFSA's CEF panel (2011).

Position on eBeam
Based on the above, it seems defendable that eBeam is outside the scope of the legislation on irradiation and therefore needs to be further assessed under the Novel Foods Regulation as set out below.

Novel foods

A potential regulatory challenge for decontamination processes, is in Regulation (EU) 2283/2015 on novel foods (a.k.a. the 'Novel Food Regulation' (NFR)). Foods that fulfil the definition of novel food may not be placed on the market in the EU except when they are authorised. For the current context it is of relevance that foods may be considered novel due to a process that has been applied.

> Article 3(2)(a) at (vii) Regulation (EU) 2283/2015 on novel foods
> 'novel food' means any food that was not used for human consumption to a significant degree within the Union before 15 May 1997, irrespective of the dates of accession of Member States to the Union, and that falls under at least one of the following categories:
> (vii) food resulting from a production process not used for food production within the Union before 15 May 1997, which gives rise to significant changes in the composition or structure of a food, affecting its nutritional value, metabolism or level of undesirable substances;

A food may come under this regulation if it results from a process that was not used in the EU before 1997 and that leads to significant changes.

First of all, it must be pointed out that it is the food that may fall under the authorisation requirement, not the process as such. This means that in case one type of food has been authorised, another type to which the same process has been applied may also need approval.

Next the process must be new. Opinions differ whether the process should be new at all or only with regard to its application to the food matrix at issue. This problem does not seem to apply here. Finally the process must lead to significant changes among other with regard to the level of undesirable substances (which probably includes undesired microorganisms). In other words, improved safety may make a food novel and thus under authorisation requirement. An issue here is the question to which benchmark the 'significant change' should be compared? If no other benchmark exists, probably the comparison must be made between the food before and the same food after treatment. It could be argued, however, that a comparison may also be made between the food treated with a conventional method and the food treated with the new method.

Processes that have a history of safe use in food production prior to 1997 are outside the scope of the NFR.

A problem with food authorisation requirements in the EU is that applications for authorisation are assed only for meeting the criteria of safety (and other criteria). No decision is given with regard to the

question whether the product is actually – in this case – a novel food. This situation somewhat limits the value of existing precedents.

High pressure processing
At least one precedent exists where (dairy) products subjected to HPP have been authorised as novel foods (European Commission Decision 2001/424, OJ 2001L 151/42 for a HPP treated yoghurt submitted by Danone). Apparently, the applicant believed that HPP would bring a food within the scope of the NFR. A later application – at that time the procedure had a first stage at Member State level which has now been abolished – has been found unnecessary by the Food Standards Agency in the UK (at that time an EU Member State). Probably the FSA considered changes of HHP (also known as cold pasteurisation) compared to pasteurisation not significant in terms of level of undesirable substances. The FSA formulated certain safety parameters such as time, temperature and pressure that must be met (for details see A. Kurowska et al. 2016).

Position on HPP
Based on the FSA precedent, it can be argued that HPP respecting relevant safety parameters can be used without prior authorisation.

Ultrasound, cold plasma, pulsed light
To the best of my knowledge, ultrasound, pulsed light and cold plasma are not methods currently used for the purpose of decontamination, and certainly not before 1997. As assessed above, it can be argued that cold plasma and pulsed light are outside the scope of the legislation on irradiation. This is certainly the case for ultrasound. These methods are intended to reduce the level of undesired microorganisms. In case comparison needs to be made with the product prior to processing, the change will undoubtedly be 'significant' (why else would you apply these processes?) and the NFR would apply. As indicated above, the HPP precedent suggests that comparison may also be made with the decontamination achieved through conventional methods. A relevant benchmark needs to be identified.

Position on ultrasound, cold plasma, pulsed light
If you can produce a relevant comparison to conventional methods of decontamination, it might be possible to argue that there are no significant changes and that these processes may be freely used.

To conclude

Conclusions
The report addresses a variety of methods for decontamination. From the analysis above, it follows that the following processes can probably be freely used:

- UV treatment
- High pressure processing
- Ultrasound
- Cold plasma
- Pulsed light
- eBeam

With regard to agents used for decontamination, the following can be concluded. Within the limits that chlorine may be present in drinking water, it can be used for the purpose of decontamination as well.

With regard to the use of substances for the purpose of decontamination, this analysis concludes that Nisin E 234 can only be used as processing aid i.e. if it does not have a function in the final product, Chlorine can be used in a concentration of 250 microgram/litre, ozone and PAA would require prior authorization.

For all substances covered in this project an application for authorization as decontaminant is worth considering.

With regard to bacteria, the analysis concludes that those lactic acid bacteria that are on the QPS list as well as *Lactobacillus reuteri* can be used for the purpose of decontamination. Bacteriophages can be used as processing aid i.e. if they do not have a function in the final product.

Recommendations
Unfortunately, my analysis leaves you with a rather high degree of uncertainty. For this reason I would recommend to seek an open exchange of views with the authorities in your EU Member State to explore 1) whether they are willing to accept the interpretations proposed, or 2) open discussion on this topic within the EU working groups in which they participate, and 3) whether they are willing to help create solutions, for example by undertaking or supporting authorisation procedures.

Businesses or business associations may consider submitting applications to the European Commission under Article 3(2) of Regulation (EC) 853/2004 for the authorisation of substances to be used as decontaminants such as chlorine (in higher concentrations than allowed in drinking water), ozone, PAA and phages.

References

Sources of law
- All EU legislation is available from the EU database of official publications 'Eur-Lex' and can easily be found through their reference number.

- Directive 1999/2/EC on foods and food ingredients treated with ionising radiation
- Directive 1999/3/EC on the establishment of a Community list of foods and food ingredients treated with ionising radiation
- Regulation (EU) 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety
- Regulation (EC) 852/2004 on food hygiene
- Regulation (EC) 853/2004 laying down specific hygiene rules for food of animal origin
- Regulation (EC) 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control
- Regulation (EC) 1333/2008 on food additives
- Regulation (EU) 2283/2015 on novel foods

Literature

- EFSA CEF Panel 2011. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), (2011)Scientific Opinion on the Chemical Safety of Food Irradiation. EFSA Journal 2011;9(4):1930. [57 pp.] doi:10.2903/j.efsa.2011.1930 www.efsa.europa.eu/efsajournal.htm;
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- University of Twente (2018) < https://www.utwente.nl/nl/hr/vg/veiligheid/straling/niet_ioniserende_straling/werken_met_niet-ioniserende_straling/ >.

12. Bilag 1 – Bakteriofager og endolysiner

Tabel 1.1. Anvendelse af bakteriofager i fødevarer og mod biofilm

Types of Food	Phage	Target pathogens	Main outcomes	Reference
Dairy products	PhageGuard Listex (Intralytix)	*L. monocytogenes*	Application of PhageGuard Listex, eradication of *Listeria* viable counts in surface-ripened red-smear soft cheese	Carlton et al. (2005)
	ListShield (Intralytix)	*L.monocytogenes*	Treatment of ListShield™, a commercially available phage cocktail Prevention of *Listeria* contamination by 0.7 log on cheese	Perera et al. (2015)
	JN01	*E. coli* O157:H7	Significant reduction of the viable *E. coli* O157:H7 in UHT milk at 4 °C	Li et al. (2021)
	vB_SauM_ME18, and vB_SauM_ME126	*S. aureus*	Elimination of MDR *S. aureus* artificially inoculated in UHT milk.	Ghareib et al. (2020)
Meats	SalmoFresh (Intralytix)	*Salmonella* spp.	Reduction of artificially inoculated *Salmonella* on Chicken breast fillets and inhibited re-growth of *Salmonella* up to 7 days at 4 °C	Sukumaran et al. (2016)
	PhageGuard S (PhageGuard)	*Salmonella* spp.	PhageGuard S, phage cocktail consisting of 2 lytic phages (S16 and F01a) Significant reduction of *Salmonella* population (1 log) on experimentally contaminated ground beef trim.	Yeh et al. (2018)
	ShigaShield (Intralytix)	*Shigella sonnei*	Prevention of *Shigella* contamination on deli meat, smoked salmon, pre-cooked chicken, lettuce, melon, and yogurt.	Soffer et al. (2017)
Vegetables	SalmoFresh (Intralytix)	*Salmonella* spp.	Application of SalmoFresh immersion to *Salmonella* spp. on Romain lettuce and sprouts Reduction of *Salmonella* on lettuce and sprouts by 2–3 log CFU/g More effective control of *Salmonella* at 2 °C and 10 °C than at 25 °C	Zhang et al. (2019)
	Ecoshield (Intralytix)	*E. coli* O157:H7	Reduction of artificially contaminated *E. coli* O157:H7 in tomatoes, broccoli, spinach, and lettuce	Abuladze et al. (2008), Sharma et al. (2009)
	ListShield (Intralytix)	*Listeria* spp.	Reduction of *Listeria* population on melon and apple slices	Leverentz et al. (2004)
	PhageGuard (Intralytix)	*Listeria* spp.	Efficient biocontrol of artificially infected *Listeria* in melon and pear juice	Oliveira et al. (2014b)
Biofilms	Listex P100	*L. monocytogenes*	Removal of biofilms formed by 13 different serotypes of *L. monocytogenes* in 3.5–5.4 log/cm²	Soni and Nannapaneni (2010)
	SalmoFresh, SalmoLyse (Intralytix)	*Salmonella* spp.	Protection of protected stainless and glass surface from *Salmonella* contamination by using SalmoFresh and SalmoLyse	Woolston et al. (2013)

Tabel 1.2. Anvendelse af endolysin i fødevarer og mod biofilm

Types of Food	Endolysin	Target pathogens	Main outcomes	Reference
Dairy products	LysH5	*S. aureus*	8-log CFU/mL bacterial reduction in milk Wide host range of clinical staphylococcal strains, including *S. aureus* and *S. epidermidis*	Obeso et al. (2008)
			Synergistic sterilization effect with nisin against *S. aureus* in milk	García et al. (2010a)
	LysSA97	*S. aureus*	Synergistic antibacterial effect in whole milk and skimmed milk	Chang et al. (2017b)
	LysSA11	*S. aureus*	2-log CFU/mL bacterial reduction in milk	Chang, Kim, and Ryu (2017a)
	Ctl1L	*C. sporogenes*	Effective but weaker antibacterial activity in milk compared to the activity in broth condition	Mayer et al. (2010)
	LysZ5	*L. monocytogenes*	4-log CFU/mL bacterial inhibition in soya milk within 3 h at refrigerator temperature (4 °C)	Zhang et al. (2012)
	Ply511, Ply118	*L. monocytogenes*	Significant reduction of viable *Listeria* cells in whole cow milk Broad host spectrum of *Listeria* strains and high thermal resistance	Schmelcher, Waldherr, and Loessner (2012b)
	Ply100	*L. monocytogenes*	Stable in cheese for up to 4 weeks Synergistic anti-listerial effect with nisin in Queso Fresco cheese	Ibarra-Sánchez, Van Tassell, and Miller (2018)
Meats	LysSA11	Multidrug-resistant *S. aureus*	3-log CFU/mL bacterial reduction in ham artificially contaminated with MRSA within 15 min at refrigerator temperature (4 °C)	Chang, Kim, and Ryu (2017a)
	Trx-SA1	*S. aureus*	Reduction of somatic cells and *S. aureus* numbers after infusion of 20 mg of Trx-SA1 in udder quarters	Fan et al. (2016)
	LysRODI	*S. aureus* *S. epidermidis*	Protective efficacy against mammary infections in mice	Gutiérrez et al. (2020)
Vegetables	LysWL59, LysWL60	*S.* Typhimurium	Reduction of 93% of *S.* Typhimurium cells on lettuce in 1 h when treated with 2.5 μM of LysWL59 and 0.5 mM EDTA	Liu et al. (2019)
	Ply511, Ply118, Ply500	*L. monocytogenes*	Reduction of viable *L. monocytogenes* cells in a spiked iceberg (Ply511, Ply118) Broad lytic spectrum against *Listeria* (Ply511)	Schmelcher, Donovan, and Loessner (2012a)
Biofilms	LysH5	*S. aureus*	Notable staphylococcal biofilm removal activity against persister cells obtained after treatment with rifampicin and ciprofloxacin	Gutiérrez et al. (2014) Gutiérrez et al. (2017)
	LysCSA13	*S. aureus*	Reduction of staphylococcal biofilms mass up to 80–90% on various food utensil surfaces, including polystyrene, stainless steel, and glass	Cha et al. (2019)
	PlyC	*S. pyogenes*	Destruction of biofilm matrixes of *S. pyogenes* which showed rapid resistance to traditional antibiotics	Shen et al. (2013)
	Lys68	*Salmonella*	Synergistic biofilm-reducing effect in combination with malic or citric acid by 1-log CFU	Oliveira et al. (2014a)
	LysPA26	*P. aeruginosa*	2–3 log reduction of viable biofilms of *P. aeruginosa* 8327 on a polystyrene plate for 48 h	Guo et al. (2017)
	PlyLM	*Listeria*	Disrupting ability against *Listeria* biofilms, Synergistic effect with a protease	Simmons et al. (2012)

Tabel 1.1 og 1.2 i Bilag 1 er kopieret fra: Lee, C., Kima, H. & S. Ryu (2022) Bacteriophage and endolysin engineering for biocontrol of food pathogens/pathogens in the food: recent advances and future trends. Critical Reviews in Food Science and Nutrition. https://doi.org/10.1080/10408398.2022.2059442.

Tabel 1.3. Bakteriofag biokontrol af fødevarebårne patogener.

Target pathogen	Description and result of the study	Reference
E. coli	100 % reduction in CFU within an hour of addition of phage DT1 and DT6 in milk during milk fermentation.	Tomat et al. 2013
	Spraying of phage cocktail on spinach blades resulted in a 4.5 log reduction of CFU after 2 h of phage addition	Patel et al. 2011
	No survivors detectable on spinach and lettuce leaves after 10 min. of phage addition combined with cinnamaldehyde treatment	Viazis et al. 2011
	Significant reduction in CFU on lettuce and cantaloupe after 2 days of spraying with phage cocktail (ECP-100)	Sharma et al. 2009
	Phage cocktail e11/2, e4/1c, pp01 applied on meat surface resulted in eradication of *E.coli* in seven of nine samples	O'Flynn et al. 2004
Campylobacter	Phage Φ29C when applied on top of chicken skin at MOI (multiplicity of infection) 1 caused less than 1 log reduction in CFU; MOI 100–1000 caused 2 log reduction in CFU	El-Shibiny et al. 2009
	Phage Cj6 was applied on top of raw and cooked beef, largest reductions were recorded at high host cell densities over a period of 8 days and incubation at 51 °C	Bigwood et al. 2009
	Phage Φ2 applied on top of chicken skin at a conc. of 10^7 PFU/ml caused 2 log reduction whereas 10^5 and 10^3 PFU/ml failed to reduce CFU count	Wagenaar et al. 2005
Salmonella	Salmonella phage F01-E2 when added to turkey deli meats and chocolate milk resulted in 5 log reduction of CFU and a 3 log reduction when applied to hot dogs	Guenther et al. 2012
	More than 99 % reduction in CFU on meat skin treated with phage cocktail PC1 at MOI 10 or above and temp 4 °C for 96 h	Hooton et al. 2011
	Combined biocontrol of phage cocktail with *Enterobacter asburiae* suppressed pathogen growth on mung beans and alfa alfa seeds	Ye et al. 2010
	Reduction of *S. javiana* in tomatoes when treated with phage and *E. asburiae* combination, although major suppressing activity was attributed to anatagonistic effect of *E. asburiae*	Ye et al. 2009
	Reduction of 3–4 log CFU in raw and cocked beef at 5 °C and 6 log CFU at 24 °C when treated with phage P7	Bigwood et al. 2009
	Phage cocktail caused significant reduction on fresh cut melons but not on apples. The result maybe explained as phage particles were inactivated due to low pH on apple surface	Leverentz et al. 2001
	No survival during 89 days in pasteurized cheeses containing phage SJ2 (MOI 10^4)	Modi et al. 2001
L. monocytogenes	Reduction of CFU by 2.5 log at 30 °C in RTE chicken. At 5 °C, regrowth was prevented over 21 days	Bigot et al. 2011
	In red smear cheese phage A511 applied on the surface caused CFU to decrease by 3 logs after 22 days. Repeated application of A511 further delayed re-growth	Guenther and Loessner 2011
	Reduction in CFU on catfish and salmon fillet upon surface application of phage P100	Soni et al. 2010
	Rapid 1 log reduction of CFU. 2 log reduction after 14 to 28 days of storage on cooked ham surface treated with Phage P100	Holck and Berg 2009
	Complete eradication of CFU on red smear soft cheese during rind washing with phage P100	Carlton et al. 2005
	Spraying melon pieces with phage cocktail after 1 h of listeria challenge reduced CFU by 6.8 log units after 7 days of storage	Leverentz et al. 2004
	Phage cocktail caused a CFU reduction of 2.0 to 4.6 log in melons and only 0.4 log in apples. Phage + nisin reduced CFU by 5.7 log in melons and 2.3 log in apple	Leverentz et al. 2003
	Phage-nisin mixture was effective in broth but not in buffer or on raw beef	Dykes and Moorhead 2002

Tabel 1.3 i Bilag 1 er tilpasset og kopieret fra: Kazi, M. & U.S. Annapure (2016) Bacteriophage biocontrol of foodborne pathogens. J Food Sci Technol 53(3):1355–1362.

Tabel 1.4. Opsummering af eksperimentelle resultater opnået ved direkte tilsætning af bakteriofager til diverse fødevarer.

Bacterium *	Phages	Notes	Ref.
Bacillus cereus	BCP1-1	*Bacillus cereus* counts decreased after treatment with a single phage in fermented soya bean paste without affecting *Bacillus subtilis*, a critical component of the fermentation process.	[20]
Campylobacter jejuni	Φ2	Counts of *Campylobacter* were reduced by ~1 log on the surface of chicken skin stored at 4 °C after the application of a single phage.	[21]
Campylobacter jejuni; Salmonella spp.	*C. jejuni* typing page 12673, P22, 29C; *Salmonella* typing phage 12	*C. jejuni* levels decreased ~2 logs on experimentally-contaminated chicken skin after application of phage at a MOI of 100:1 or 1000:1. *Salmonella* levels were reduced by ~2 logs on chicken skin treated with phage at an MOI of either 100:1 or 1000:1 and stored for 48 h; bacterial counts were reduced below the limit of detection when lower levels of bacteria were used to contaminate the chicken.	[22]
Campylobacter jejuni; Salmonella spp.	Cj6; P7	*Campylobacter* levels significantly decreased in beef after application of the phage Cj6, and decreases in bacterial levels were not significant at low levels of bacterial contamination (~100 CFU/cm^2). *Salmonella* counts were decreased ~2–3 logs at 5 °C and >5.9 logs at 24 °C in raw and cooked beef after P7 phage application. Surviving *Salmonella* colonies were still sensitive to P7. For both phages, the killing of bacteria was higher at an MOI of 10,000:1 and ~10,000 CFU/cm^2 of bacteria.	[23]
Cronobacter sakazakii	ESP 1-3, ESP 732-1	In infant formula, *Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) levels were decreased after phage addition. The reduction was dependent on the phage concentration, and the phages were more effective at 24 °C than 37 °C or 12 °C.	[24]
Cronobacter sakazakii	Five phages	Growth of 36 of 40 test strains was inhibited by a phage cocktail tested in infant formula experimentally contaminated with *C. sakazakii*. Furthermore, both high and low concentrations (10^6 and 10^2 CFU/mL) of bacteria were eliminated from liquid culture medium treated with the individual phage (10^8 PFU/mL).	[25]
Escherichia coli O157:H7	e11/2, pp01, e4/1c	After incubation at 37 °C, a three-phage cocktail used to treat the surface of beef that was contaminated (10^3 CFU/g) with *E. coli* O157:H7 eliminated the bacterium from a majority of the treated specimens.	[26]
Escherichia coli O157:H7	EcoShield™ (formerly ECP-100)	*E. coli* 0157:H7 levels decreased by ~1–3 logs, or were reduced below the limits of detection, on tomatoes, broccoli or spinach after treatment with a phage cocktail, while *E. coli* O157:H7 levels were decreased by ~1 log when the phages were applied to ground beef.	[17]
Escherichia coli O157:H7	EcoShield™ (formerly ECP-100)	A phage cocktail applied to experimentally contaminated lettuce and cut cantaloupe significantly reduced *E. coli* O157:H7 levels by up to 1.9 and 2.5 logs, respectively.	[27]
Escherichia coli O157:H7	Cocktail BEC8	At various temperatures (4, 8, 23 and 37 °C), the phage cocktail significantly reduced the level of *E. coli* O157:H7 on leafy green vegetables by ~2–4 logs. The inclusion of an essential oil (*trans*-cinnamaldehyde) increased this effect.	[28]
Escherichia coli O157:H7	EcoShield™ (formerly ECP-100)	The levels of *E. coli* O157:H7 were reduced by ≥94% and ~87% on the surface of experimentally contaminated beef and lettuce, respectively, after addition of the phage cocktail; however, the single treatment did not protect foods after recontamination with the same bacteria (i.e., phage biocontrol had no continued technical effect on the foods).	[29]
Escherichia coli O157:H7	EcoShield™ (formerly ECP-100)	After a 30 min phage treatment at both 4 and 10 °C, levels of *E. coli* O157:H7 decreased by >2 logs on leafy greens under both ambient and modified atmosphere packaging storage.	[30]

Tabel 1.4. (forts.)

Bacterium *	Phages	Notes	Ref.
Escherichia coli	FAHEc1	Contamination of raw and cooked beef decreased by 2–4 logs at 5, 24 and 37 °C in a concentration dependent manner after phage application. The *E. coli* displayed regrowth at higher temperatures.	[31]
Escherichia coli O157:H7	EcoShield™ (formerly ECP-100)	A phage cocktail was applied to lettuce by spraying and dipping. A larger initial reduction (~0.8–1.3 logs) in *E. coli* O157:H7 counts was observed after spraying. Dipping required submerging the lettuce for as long as 2 min, and the initial reductions were not significant. After 1 day of storage at 4 °C, dipping in the highest concentration of the phage cocktail reduced *E. coli* by ~0.7 log.	[32]
Escherichia coli	EC6, EC9, EC11	Two *E. coli* strains were eradicated from raw and UHT milk after treatment with a three-phage cocktail at 5–9 °C and 25 °C. For a third *E. coli* strain, phage treatment eliminated the bacteria from UHT milk; however, after an initial reduction, regrowth occurred in the raw milk after 144 or 9 h for 5–9 °C and 25 °C storage, respectively.	[33]
Escherichia coli, Salmonella, Shigella	EcoShield™ (formerly ECP-100), SalmoFresh™, ShigActive™	Phage cocktails were as effective or more effective than chlorine wash at reducing targeted pathogenic bacteria from broccoli, cantaloupe and strawberries in samples containing a large amount of organic content. Combination of the phage cocktail and a produce wash generated a synergistic effect, i.e., higher reductions of bacteria.	[34]
Listeria monocytogenes	ListShield™ (formerly LMP-102)	*Listeria* counts decreased by ~2 logs and ~0.4 log after application of a phage cocktail on melon and apple slices, respectively; a synergistic effect was observed when phage and nisin were used, decreasing levels of *Listeria* on the fruit ~5.7 logs and ~2.3 logs, respectively.	[35]
Listeria monocytogenes	ListShield™ (formerly LMP-102)	Application of a phage cocktail 1, 0.5 or 0 h before honeydew melon tissue were contaminated with the bacterium was most effective at reducing *Listeria* counts. This effect depended on the concentration of phage applied. *Listeria* counts decreased by ~5–7 logs after 7 days, when the phages were applied at the times described above.	[36]
Listeria monocytogenes	PhageGuard Listex™ (formerly Listex™; P100)	Levels of *Listeria* were reduced by at least 3.5 logs after a single phage was administered to the surface of ripened red-smear soft cheese. The surviving *Listeria* colonies isolated from the cheese after phage treatment were not resistant to the phage.	[37]
Listeria monocytogenes	A511, PhageGuard Listex™ (formerly Listex™; P100)	Levels of *Listeria* in experimentally contaminated chocolate milk and mozzarella cheese brine were eradicated after phage treatment at 6 °C. When the phage cocktail was applied to various solid foods, including sliced cabbage, iceberg lettuce leaves, smoked salmon, mixed seafood, hot dogs, and sliced turkey meat, a reduction of *Listeria* of up to 5 logs was observed.	[38]
Listeria monocytogenes	PhageGuard Listex™ (formerly Listex™; P100)	*Listeria* counts decreased by 1.8–3.5 logs after application of a single phage at ~10^8 PFU/g to the surface of raw salmon fillets that were stored at 4 °C or 22 °C.	[39]
Listeria monocytogenes	PhageGuard Listex™ (formerly Listex™; P100)	Levels of *Listeria* decreased by 1.4–2.0 logs CFU/g at 4 °C, 1.7–2.1 logs CFU/g at 10 °C, and 1.6–2.3 logs CFU/g at room temperature (22 °C) after application a single phage to the surface of raw catfish fillets. Regrowth was not observed after ten days of storage at either 4 °C or 10 °C.	[40]

Tabel 1.4. (forts.)

Bacterium *	Phages	Notes	Ref.
Listeria monocytogenes	A511	The natural microbial community on soft cheese was maintained after addition of the phage. Levels of *Listeria* on experimentally contaminated cheese decreased by 2 logs and additional phage administrations did not improve the reduction of *Listeria*.	[41]
Listeria monocytogenes	FWLLm1	*Listeria* levels decreased by 1–2 logs on the surface of experimentally contaminated chicken stored in vacuum packages at 4 °C or 30 °C. Subsequent regrowth of *Listeria* was observed at 30 °C, but not at 4 °C.	[42]
Listeria monocytogenes	PhageGuard Listex™ (formerly Listex™; P100)	Counts of *Listeria* decreased by ~3 logs in experimentally contaminated queso fresco cheese after the addition of a single phage; however, subsequent growth was observed. Regrowth was prevented, and a similar log reduction was observed when PL + SD were included with the phage. Reduction of *Listeria* was lower, and regrowth occurred when LAE was included with phage.	[43]
Listeria monocytogenes	PhageGuard Listex™ (formerly Listex™; P100)	Compared to PL or PL + SD, a single phage was most effective at decreasing *Listeria* levels on RTE roast beef and turkey after storage at 4 °C or 10 °C, and subsequent bacterial growth was observed at both temperatures. Similar log reductions occurred when PL or PL + SD were used in conjunction with the phage, and regrowth was prevented or diminished at both 4 °C and 10 °C.	[44]
Listeria monocytogenes	PhageGuard Listex™ (formerly Listex™; P100)	Counts of *Listeria* decreased by ~1.5 logs on experimentally contaminated melon and pear slices, but not apple slices after two days at 10 °C. Additionally, treatment with phage did not impact *Listeria* levels in apple juice but decreased bacterial contamination by ~4 and ~2.5 logs in melon and pear juice, respectively.	[45]
Listeria monocytogenes	PhageGuard Listex™ (formerly Listex™; P100)	*Listeria* levels on soft cheese decreased by ~2–3 logs after 30 min and ~0.8–1 log after storage for 7 days at 10 °C.	[46]
Listeria monocytogenes	ListShield™ (formerly LMP-102)	Counts of *Listeria* decreased by 0.7 and 1.1 log on experimentally contaminated cheese and lettuce, respectively, after a 5 min treatment with phage and decreased the bacteria 1.1 log on the surface of apple slices after 24 h when combined with an antibrowning solution. The phage cocktail also virtually eliminated *Listeria* from experimentally contaminated frozen entrees that were frozen and thawed after treatment and was effective at eliminating environmental contamination by *Listeria* at a smoked salmon preparation plant.	[10]
Listeria monocytogenes	PhageGuard Listex™ (formerly Listex™; P100)	When applied to the surface of experimentally contaminated sliced pork ham, the phage reduced *Listeria* counts below the limit of detection after 72 h, and performed better than nisin, sodium lactate, or combinations of these antibacterial measures.	[47]
Mycobacterium smegmatis	Six phages	*M. smegmatis* counts were reduced below the limit of detection in milk treated with a six-phage cocktail or each component phage. Subsequent bacterial growth occurred when the component phages were used, but no growth was observed after 96 h at 37 °C, when the cocktail was applied.	[48]
Salmonella spp.	SJ2	*Salmonella* levels were reduced by 1–2 logs in raw and pasteurized cheeses created using milk that was treated with phage, while cheese made from milk without phage saw *Salmonella* counts rise ~1 log.	[49]

Tabel 1.4. (forts.)

Bacterium *	Phages	Notes	Ref.
Salmonella spp.	SCPLX-1	Counts of *Salmonella* decreased by ~3.5 logs at 5 and 10 °C and ~2.5 logs at 20 °C on melon slices after application of a four-phage cocktail; treatment of apple slices with phage showed no reduction of bacteria.	[50]
Salmonella spp.	Felix-O1	*Salmonella* counts decreased by 1.8–2.1 logs after phage application to chicken frankfurters.	[51]
Salmonella spp.	PHL4	The levels of *Salmonella* recovered from experimentally contaminated broiler and naturally contaminated turkey carcasses were reduced by as high as 100% or 60%, respectively, after phage administration.	[52]
Salmonella spp.		Levels of *Salmonella* decreased by ~3 logs after application of a phage cocktail to sprouts; addition of an antagonistic bacteria to the phage cocktail increased this reduction to ~6 logs.	[53]
Salmonella spp.	FO1-E2	In chocolate milk and mixed seafood, *Salmonella* levels were reduced to undetectable levels after phage treatment and storage for 24 h at 8 °C and remained below the limit of detection. When foods were phage-treated and stored at 15 °C, *Salmonella* counts were reduced to undetectable levels within 24–48 h for hot dogs, sliced turkey breast, and chocolate milk, but regrowth occurred after 5 days. *Salmonella* levels were initially inhibited at ~0.5–2 logs and ~1–3 logs in egg yolk and mixed seafood, respectively, after phage addition; but bacterial recovery matched controls in egg yolks after two days, while the log reduction was maintained in seafood.	[54]
Salmonella spp.	UAB_Phi 20, UAB_Phi78, UAB_Phi87	*Salmonella* counts decreased by ~1 log on the shells of fresh eggs and by 2–4 logs on lettuce 60 min after application of the phage. After an initial reduction of 1–2 logs, when chicken breasts were dipped in a phage cocktail, no further decrease in the bacterial counts was observed over the next seven days at 4 °C. The levels of *Salmonella* were reduced by 2–4 logs on pig skin after phage application and storage for 6 h at 33 °C.	[55]
Salmonella spp.	wksl3	*Salmonella* counts decreased by ~3 logs on chicken skin after application of a single phage, and no further decrease in bacterial levels was observed over the next seven days at 8 °C. Further, mice that received a single dose of phage orally displayed no adverse effects.	[56]
Salmonella spp.	Five phages	The levels of *Salmonella* decreased by ~1 log on chicken skin after application of a five-phage cocktail comprised of closely related phages. The reduction of bacteria achieved by the phages was comparable to three different chemical antimicrobials.	[57]
Salmonella spp.	P22	After the administration of a single temperate phage and storage at 4 °C, levels of *Salmonella* decreased by ~0.5–2 logs on chicken, below the limits of detection in whole and skimmed milk, ~3 logs in apple juice, ~2 logs in liquid egg, and ~2 logs in an energy drink.	[58]
Salmonella spp.	SalmoFresh™	The stability of a *Salmonella*-specific phage preparation was determined in various chemical antimicrobials. Treatment of chicken breast fillets with a combination of phages and individual chemical antimicrobials did not produce a synergistic effect on the reduction of *Salmonella*; however, application of chlorine or PAA followed by spraying with phages significantly reduced *Salmonella* from chicken skin by up to 2.5 logs, compared to use of chlorine, low levels of PAA, or phage alone (0.5–1.5 logs).	[59]

Tabel 1.4. (forts.)

Bacterium *	Phages	Notes	Ref.
Salmonella spp.	SalmoFresh™	Treatment of chicken breast fillets by dipping or surface application of a *Salmonella*-specific bacteriophage preparation and storage at 4 °C significantly reduced *Salmonella* contamination by up to 0.9 log; further, storing the meat in modified atmospheric packaging after surface application produced a higher reduction in bacterial counts (up to 1.2 logs).	[60]
Salmonella spp.	SalmoLyse®	A phage cocktail was sprayed onto experimentally contaminated raw pet food ingredients, including chicken, tuna, turkey, cantaloupe, and lettuce, and reduced the levels of the targeted bacteria by ~0.4–1.1 logs.	[61]
Salmonella spp.	SJ2	Application of the phage SJ2 significantly reduced *Salmonella* colonies recovered from experimentally contaminated ground pork and eggs with a larger reduction observed at room temperature, compared to 4 °C. After treatment, *Salmonella* colonies were screened for phage resistance, and significantly more phage-resistant *Salmonella* isolates were recovered from eggs, compared with ground pork.	[62]
Salmonella spp.	PhageGuard S™ (formerly Salmonelex™)	Boneless chicken thighs and legs were experimentally contaminated with *Salmonella* serovars isolated from ground chicken or other sources. A larger reduction of *Salmonella* was achieved when the bacteriophage preparation was diluted in tap water, compared to filtered water prior to application, and the phage cocktail was more effective against *Salmonella* isolated from other sources, compared to those from ground chicken.	[63]
Salmonella spp.	PhageGuard S™ (formerly Salmonelex™)	Treatment with a bacteriophage cocktail or irradiation significantly reduced (~1 log) the level of *Salmonella* on experimentally contaminated ground beef trim, and a combination of these methods decreased bacterial contamination by ~2 logs.	[64]
Shigella spp.	SD-11, SF-A2, SS-92	*Shigella* counts were reduced by ~1–4 logs on pieces of spiced chicken after application of a phage cocktail or each of the component phages and storage at 4 °C.	[65]
Shigella sonnei	ShigaShield™	Application of a five-phage, *Shigella*-specific cocktail to various RTE foods, including lettuce, melon, smoked salmon, corned beef and pre-cooked chicken, reduced the recovery of *Shigella* ~1.0–1.4 logs at the highest phage concentration applied compared to control.	[66]
Staphylococcus aureus	Φ88, Φ35	*S. aureus* levels decreased below the limit of detection in experimentally contaminated whole milk after treatment, with a two-phage cocktail and storage at 37 °C. After phage treatment, *S. aureus* was not recovered from the acid curd after storage for 4 h at 25 °C, and was eliminated from the renneted curd after 1 h at 30 °C.	[67]
Staphylococcus aureus	vB_SauS-phi-IPLA35, vB_SauS-phi-SauS-IPLA88	Counts of *S. aureus* were significantly decreased in cheese made using milk treated with phages compared to milk without the addition of phages. The microbiota of the cheese was not impacted by the addition of the phages.	[68]

* Listed in alphabetical order by bacteria and then chronologically. In cases where multiple bacteria were examined, the study is listed with the alphabetically first bacteria. Adapted and modified from Sulakvelidze 2013 and Woolston and Sulakvelidze 2015 [12,18]. h, hour; LAE, lauric arginate; log(s), logarithmic unit(s); min, minutes; PAA, peracetic acid; PL, potassium lactate; PL + SD, potassium lactate + sodium diacetate; RTE, Ready-To-Eat; UHT, ultra-high temperature.

Tabel 1.4 i Bilag 1 er kopieret fra: Moye, Z.D., Woolston, J. & A. Sulakvelidze (2018) Bacteriophage Applications for Food Production and Processing. Viruses 10, 205.

Tabel 1.5. Kommercielle produkter der indeholder bakteriofager og parametre for brug af disse.

Host	Bacteriophage	Dose	Treatment time	Matrix	Reduction log	References
Listeria monocytogenes	LISTEX™ P100	10^7 PFU/cm^2	30 min, 1, 2, 3, 7, 10, 14, 20, and 28 days	Roast beef and cooked turkey	2 $\log_{10}$ CFU/cm^2	Chibeu et al. (2013)
	FWLLm1	2.5×10^7 PFU/cm^2	24 h	Ready-to-eat chicken breast roll	2.5 $\log_{10}$ CFU/cm^2	Bigot et al. (2011)
	P100/A511	3×10^8 PFU/g	6 days	Hot dogs (sausages), cooked and sliced turkey breast meat (cold cuts), smoked salmon, mixed seafood (cooked and chilled cocktail of shrimp, mussels, and calamari), chocolate milk (pasteurized, 3.5% fat), mozzarella cheese brine (unsalted pasteurized whey from plastic bag containers containing fresh mozzarella cheese), iceberg lettuce (leaves), and cabbage (sliced fresh leaves)	1–3 $\log_{10}$ CFU/cm^2	Guenther et al. (2009)
	ListShield™	10^9 PFU/mL	0, 2, 5, and 7 days	Fresh-cut melons and apples	3.5 $\log_{10}$ CFU/cm^2	Leverentz et al. (2001)
Salmonella nteritidis	SJ2	10^8 PFU/mL	24 h	Raw and pasteurized milk cheeses	1–2 $\log_{10}$ CFU/cm^2	Modi et al. (2001)
	PHL 4	10^{10} PFU/mL	24 h	Poultry carcass	3 $\log_{10}$ CFU/mL	Higgins et al. (2005)
Salmonella typhimurium	Felix-O1	5.25×10^6 PFU	24 h	Chicken frankfurters	2 $\log_{10}$ CFU/g	Whichard et al. (2003)
	SalmoFresh	10^8 PFU/mL	5 h	Ready-to-eat chicken products	2 $\log_{10}$ CFU/mL	
S. enteritidis and *S. typhimurium*	wksl3	2.2×10^8 PFU/mL	1, 2, 3, 5, and 7 days	Chicken skin	3 $\log_{10}$ CFU/mL	Kang et al. (2013)
	SalmoFREE	10^8 PFU/mL	36 days	*In vivo*–chicken production	3 $\log_{10}$ CFU	Clavijo et al. (2019)
	Salmonelex™	10^9 PFU/mL	24 h	Ground beef and ground pork	1.1 and 0.9 $\log_{10}$ CFU/g	Yeh et al. (2017)

Tabel 1.5 i Bilag 1 er kopieret fra: Rogovski, P., Cadamuro, R.D., da Silva, R., de Souza, E.B., Bonatto, C., Viancelli, A., Michelon, W., Elmahdy, E.M., Treichel, H., Rodríguez-Lázaro, D. & G. Fongaro (2021) Uses of Bacteriophages as Bacterial Control Tools and Environmental Safety Indicators. Front. Microbiol. Vol. 12, Art. 793135.

13. Bilag 2 – Diverse ikke-termiske teknologier – fisk/skaldyr

Tabel 2.1. Effekt af ikke-termiske teknologier på *Salmonella* spp. og *Escherichia coli* niveauer.

Methodology (*Microoganisms*)	Fish species	Treatment/Reduction (log CFU/g)	Reference
Irradiation			
(S. enteritidis)	Oyster	3.0 kGy/5.0–6.0 log	Jakabi et al. 2003
(Salmonella spp.)	Shrimp	6.0 kGy/7.0 log	Abreu et al. 2009
(S. enteritidis)	Shrimp	3.0 kGy/6.0 log	Mahmoud 2009
(Salmonella spp.)	Rainbow Trout	5.0 kGy/4.0 log	Oraei et al. 2011
(Salmonella spp.)	Mackerel	3.0 kGy/6.0 log	Acharjee et al. 2014
(Salmonella spp.)	Poa	3.0 kGy/6.0 log	Acharjee et al. 2014
(Salmonella spp.)	Frozen Mullet	5.0 kGy/5.0 log	Aly et al. 2014
(E. coli)	Squid	3–5 kGy/2.0–3.0 log	Manjaniak et al., 2018
HPP			
E. coli	Black Tiger Shrimp	100 Mpa, 5 min/0.41 log	Kaur et al. 2013
	Black Tiger Shrimp	270 MPa, 5 min/1.20 log	Kaur et al. 2013
	Black Tiger Shrimp	435 Mpa, 5 min/1.53 log	Kaur et al. 2013
Pulsed light			
(E. coli)	Salmon fillet	5.6 J cm^{-2}/0.86–1.09 log	Ozer and Demirci 2006
Ultraviolet			
(E. coli)	Sliced Squid	UV-C 253.7 nm, 1–30 min/1.35 log	Lee et al. 2016
(E. coli)	Threadfin Bream	UV-C 253.7 nm, 20 min/1.1 log	Omaima and Hesham 2019
(E. coli)	Tilapia	UV-C 253.7 nm 15 min/1.82 log	Omaima and Hesham 2019
Ozone			
(E. coli)	Oyster	0.6 mg/L/1.3 log	López Hernandez et al. 2018

Tabel 2.2. Effekt af ikke-termiske teknologier på *Listeria* spp. niveauer.

Methodology(*Microorganisms*)	Fish species	Treatment/Reduction (log CFU/g)	Reference
Irradiation			
Listeria monocytogenes	Salmon fillets	1.0 kGy/2.5 log	Su et al. 2004
Listeria spp.	Mackerel	6.0 kGy/4.0 log	Acharjee et al. 2019
Listeria spp.	Koral fish	6.0 kGy/4.0 log	Acharjee et al. 2019
HPP			
Listeria innocua	Minced trout	414 MPa-5 min/4.0 log	Akgul et al. 2010
Listeria monocitogenes	Mussel meat	400 MPa/5.0 log	Fletcher et al. 2008
Pulsed light			
Listeria monocytogenes	Salmon fillet	5.6 J/cm^2/pulse/0.74–1.02 log	Ozer and Demirci 2006
Listeria monocytogenes	Shrimp fillet	1.75 J/cm^2/pulse/2.2–2.4 log	Cheigh et al. 2013
Listeria monocytogenes	Salmon fillet	1.75 J/cm^2/pulse/1.9–2.1 log	Cheigh et al. 2013
Listeria monocytogenes	Flatfish fillet	1.75 J/cm^2/pulse/1.7–1.9 log	Cheigh et al. 2013
Listeria monocytogenes	Tuna carpaccio	8.4–11.9 J/cm^2/0.2–0.7 log	Hierro et al. 2012
	Raw salmon	Broad-spectrum light pulses (200–1000 nm), power 1516 W, pulse width 360 µs with a fixed pulse rate of 3 pulses/sec (Hz), duration 9 sec/ 1.5 log	Pedros-Garrido et al., 2018
Ultraviolet			
Listeria monocytogenes	Raw salmon fillets	Short-wave ultraviolet (UVeC) light (254 nm) duration 5–10 min/0.6 log	Mikš-Krajnik et al. 2017
	Raw salmon	Broad-spectrum light (200–1000 nm), power 1516 W, duration 9 sec/1.5 log	Pedros-Garrido et al., 2018
Ozone			
(Listeria innocua)	Salmon-trout	0.1×10^{-3} g/L, duration 20 min/1.0 log	Vaz-Velho et al. 2006
(Listeria innocua)	Salmon-trout fillets	0.1×10^{-3} g/L, duration 20 min/0.2 log	Vaz-Velho et al. 2006
(Listeria innocua)	Salmon fillets	1.0 and 1.5 mg/L ozone in aqueous sprays, three spray passes/1.17 log	Crowe et al. 2012
(Listeria innocua)	Shrimp	9.5 L/min of ozonated water, spray duration 55–65 sec/1–2 log	Guo et al. 2013

Tabel 2.1 og 2.2 i bilag 2 er kopieret fra: Andoni, E., Ozuni, E., Bijo, B., Shehu, F., Branciari, R., Miraglia, D. & D. Ranucci (2021) Efficacy of Non-thermal Processing Methods to Prevent Fish Spoilage. Journal of Aquatic Food Product Technology. Vol. 30,2:228-245.

14. Bilag 3 - Diverse ikke-termiske teknologier – kød/fisk/skaldyr

Tabel 2.5. Effekt af ultralyd, HPP, gammabestråling og UV-C bestråling i reducering af patogene og fordærvende mikroorganismer i kød- og fiskeprodukter.

NTPT	Meat and fish matrix	Microorganism	Decimal reduction (log cfu) and other antimicrobial effects	Conditions of application	Reference
US	Salmon	*Pseudomonas* spp.	1.3/g	30 kHz, 45 min	Pedrós-Garrido et al. (2017)
	Mackerel		0.7/g		
	Cod		0.6/g		
	Hake		0.6/g		
US	Meat model system	*Lactobacillus sakei*	Increase of 15.21 h in lag phase (λ, h)	20 kHz, 49.1 W, 7 min	Ojha et al. (2016a)
US	Beef slurry	*Clostridium perfringens* spores	3.0 log reduction for spores. Combined heat and US reduce the application time by half to reach the same effect to isolated heat treatment	Heat: 95 °C, 30 min US: 24 kHz, 460 W/cm^2	Evelyn and Silva (2015)
US	Beef extract	Total coliforms	2.2/mL	40 kHz, 11 W/cm^2, 90 min	Caraveo et al. (2015)
		Mesophilic bacteria	2.91/mL		
		Psychrophilic bacteria	3.18/mL		
US	Raw salmon	*L. monocytogenes*	0.35/g	Ultrasound of bath, 45 kHz, 200 W, 1 min	Mikš-Krajnik et al. (2017)
	Fillets	Coliforms	0.28/g		
HPP	Beef	Mesophilic bacteria	2.5/g	300 Mpa, 5 min	Giménez et al. (2015)
		Lactic acid bacteria	1.5/g		
HPP	Chicken breast fillet	*E. coli*	1.69/g	300 Mpa, 5 min	Kruk et al. (2011)
		S. Typhimurium	0.64/g		
		L. monocytogenes	3.22/g		
HPP	Poultry	Mesophilic bacteria	1.52/g	300 Mpa, 10 min	Canto et al. (2015)
		Psychrotrophic bacteria	>2.38/g		
HPP	Poultry sausages	*Brochothrix thermosphacta*	3.5/g	350 Mpa, 120 s, counting performed 20 ± 2 h after the treatment	Al-Nehlawi et al. (2014)
		Campylobacter jejuni			
		Leuconostoc carnosum	>6.0/g		
		L. innocua	0.5/g		
		S. Enteritidis	0.5/g		
			3.5/g		
HPP	Poultry	*C. jejuni*	0.04/g	200 Mpa, 5 min	Jackowska-Tracz and Tracz (2015)
			2.70/g	300 Mpa, 5 min	
			>6.97/g	400 Mpa, 5 min	
GI	Shrimp	Mesophilic bacteria	2.65/g	2.5 kGy	Mahto et al. (2015)
		Yeasts and molds	2.33/g		
		Coliform counts	>3.75/g		
GI	Semi-dried squid	Murine norovirus strain MNV-1	0.6 pfu/mL	3 kGy	Kang, Park, et al. (2016)
			0.9 pfu/mL	5 kGy	
			1.4 pfu/mL	7 kGy	
			1.8 pfu/mL	10 kGy	
GI	Dry fermented pork sausages	Total plate counts	0.85/g	0.5 kGy	Kim et al. (2012)
			1.11/g	1.0 kGy	
			0.98/g	2.0 kGy	
			3.88/g	4.0 kGy	
GI	Shrimp	*S. aureus*	>3.45/g	3 kGy	Hocaoğlu et al. (2012)
		E. coli	>3.86/g		
		Mesophilic bacteria	2.79/g		
		Coliform counts	2.82/g		
GI	Ground beef	Mesophilic bacteria	2.58/g	2 kGy	Ayari et al. (2016)
		Psychrotrophic bacteria	3.76/g		
		Yeasts and molds	1.32/g		
GI	Shrimp	*Bacillus subtilis*	1.5/g	3 kGy	Wang et al. (2010)
		E. coli	6.8/g		
UV	Chicken breast	Murine norovirus-1 (MNV-1)	1.23 PFU/mL	3600 mWs/cm^2	Park and Ha (2015)
		Hepatitis A virus (HVA)	1.17 PFU/mL		
UV	Fish fillets (rainbow trout)	Mesophilic bacteria	Prolongation of lag phase	106.32 mJ/cm^2	Rodrigues et al. (2016)
		Psychrotrophic bacteria	The increased microbial growth rate		
		Enterobacteria			
UV	Bullfrog shredded	*S. aureus*	3.19/g	1.68 mWs/cm^2, 140 s	Silva et al. (2015)
UV	Chicken	*Salmonella* strains	0.57/g	1.95 mW/cm^2, 120 s	Lázaro et al. (2014)
UV	Fish	Mesophilic bacteria	0.26/g	55.83 mJ/cm^2	Bottino et al. (2017)
		Psychrophilic bacteria	0.25/g		
		Enterobacteriaceae	0.15/g		
UV	Bologna Beef	*E. coli*	4.6/mL	164 mJ/cm^2	Tarek, Rasco, and Sablani (2015)

NTPT: Non-thermal preservation technology, US: Ultrasound, HPP: High pressure processing, GI: Gamma irradiation, UV: UV-C radiation.

Tabel 2.6. Effekt af ultralyd, HPP, gammabestråling og UV-C bestråling fysisk/kemiske og sensoriske karakteristika i kød- og fiskeprodukter.

Table 2. Effects of ultrasound, high pressure processing, gamma irradiation and UV-C radiation on the physicochemical and sensory characteristics of meat and fish products.

NTPT	Meat and fish matrix	Effects on matrix	Condition of application	Reference
US	Pork	Softening	20 kHz, 750 W, 120 min 54.9 W/cm^2	Ojha et al. (2016b)
US	Chicken breast meat batter	Improved of textural properties (hardness, springiness, cohesiveness, chewiness and gel strength) and water holding capacity (WHC)	40 kHz, 300 W, 20 min	Li et al. (2015)
US	Beef extract	Increased of *L* value (luminosity) and pH reduction. No changes in redness and yellowness, as well as WHC and drip loss	40 kHz, 11 W/cm^2, 60 and 90 min	Caraveo et al. (2015)
US	Beef	Increased of lipid and protein oxidation dependent of the increased ultrasonic power	20 kHz, 2.39, 6.23, 11.32 and 20.96 W/cm^2, 120 min	Kang, Zou, et al. (2016)
US	Beef	Decreased of hardness and gumminess. No changes in L^*, $a^*$and b^* values	40 kHz, 1,500 W, 10 min	Chang et al. (2012)
HPP	Squid muscles	Low changes in volatile compounds and no changes in total free amino acids	200 e 400 Mpa, 10 min	Yue et al. (2016)
HPP	Chicken breast fillet	Reduced of flavor and juiciness. Increased cooking loss, L^* and b^* values, cohesiveness, gumminess and lipid oxidation. The higher the pressure applied the greater the changing.	300, 450 and 600 Mpa, 5 min	Kruk et al. (2011)
HPP	Goat meat	Increased pH, springiness and L^* and b^* values. Decreased of a^* values	300 e 600 Mpa, 5 e 10 min	Jalarama Reddy et al. (2015)
GI	Fish	No undesirable sensory changes	1.5 kGy	Monteiro et al. (2013)
GI	Semi-dried squid	No changes in sensory, color and lipid oxidation	3, 5, 7 and 10 kGy	Kang, Zou, et al. (2016)
GI	Fish	Reduced of histamine and increased lipid oxidation	1 and 3 kGy	Maltar-Strmečki et al. (2013)
GI	Ground beef	Increased of lipid oxidation (peroxide values)	2 kGy	Ayari et al. (2016)
GI	Shrimp	No changes in shear force and toughness after irradiation application. Improved of visual appearance with doses greater than 2.5 kGy	0.5, 1.5, 2.5, 5 and 10 kGy	Mahto et al. (2015)
GI	Chicken sausage	No changes in pH, odor, color and sensory acceptance	2.5 and 5.0 kGy	Hwang et al. (2015)
UV	Chicken breast	The higher the dose the higher the lipid oxidation index and a^* value, whereas the lower is L^*value With the increase of the dose occurs reduction of the notes given by evaluators for the attributes of color, flavor, texture, appearance, overall sensory acceptability	60-3600 mWs/cm^2	Park and Ha (2015)
UV	Beef	Reduced of rightness, yellowness and redness values. No changes in pH, shear force and volatile basic nitrogen	4.5 mW/cm^2, 20 min	Kim, Lee, and Eun (2015)
UV	Fish fillets (rainbow trout)	No change in lipid oxidation values and high initial concentrations of biogenic amines	106.32 mJ/cm^2	Rodrigues et al. (2016)
UV	Chicken	Increased contents of tyramine	1.13 e 1.95 mW/cm^2, 90 s	Lázaro et al. (2014)
UV	Fish	No change in lipid oxidation values and increased of biogenic amines values,	160.97 mJ/cm^2	Bottino et al. (2017)
UV	Fish fillets (Nile Tilapia (*Oreochromis niloticus*))	Increased histamine, cadaverine and putrescine initial values with higher doses application	0.103 and 0.305 J/cm^2	Monteiro et al. (2017)

NTPT: Non-thermal preservation technology, US: Ultrasound, HPP: High pressure processing, GI: Gamma irradiation, UV: UV-C radiation.

Tabel 2.5 og 2.6 i bilag 3 er kopieret fra: Rosario, D. K. A., Rodrigues, B. L., Bernardes, P. C. & C. A. Conte-Junior (2021) Principles and applications of non-thermal technologies and alternative chemical compounds in meat and fish. Critical Reviews in Food Science and Nutrition. 61,7:1163-1183.

15. Bilag 4 – Bestråling – kød/fisk/skaldyr

Tabel 2.3. Holdbarhed af udvalgte hakket oksekød og fjerkræ produkter som funktion af pakkeforhold og eBeam behandling.

Meat product	Packaging atmosphere	Shelf-life (days)		
		Non-MAP	MAP-non irradiated	MAP-irradiated
Fresh ground beef	High oxygen	2–3	7–11	Not applicable
Fresh ground beef	Low oxygen	2–3	14–21	30–31
Fresh ground beef	Non-MAP	2–3	Not applicable	22–28
Beef cuts	Vacuum	25–30	Not applicable	47
Fresh ground beef chubs	Chub film	14–20	Not applicable	≥34
Skinless/boneless poultry	Case ready	3–9	11–13	~30

Tabel 2.3 i bilag 4 er kopieret fra: Pillai, S. D. & S. Shayanfar (2017) Electron Beam Technology and Other Irradiation Technology Applications in the Food Industry in Applications of Radiation Chemistry in the Fields of Industry, Biotechnology and Environment, pp. 249-268. Springer Verlag.

Tabel 2.4. Effekt af bestråling af kødprodukter.

Microorganism	Source	Dose rate for 5$\log_{10}$ reduction	Reference
L. monocytogenes in RTE meat meals[a]	Gamma	2.45–3.75 kGy	Sommers et al. (2004)
E. coli (KCTC) in marinated beef rib	Gamma	3.0 kGy	Jo et al. (2004)
Salmonella spp. in rabbit meat	Gamma	3.0 kGy	Bard (2005)
FBP[b] in cured dry ham	E-beam	5.0–5.4 kGy	Cava et al. (2005)
FBP[b] in loins			Carrasco et al. (2005)
FBP[b] in RTE meal meals[c]	Gamma	1.8–3.0 kGy	Sommers and Boyd (2006)

[a] Frankfurter, bologna pasta, ham and deli turkey meat.
[b] *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, and *Salmonella* spp.
[c] Frankfurter, beef cheeseburger and vegetarian cheeseburg.

Tabel 2.4 i bilag 4 er kopieret fra: Aymerich, T., Picouet, P. A. & J. M. Monfort (2008) Decontamination technologies for meat products. Meat Science Vol. 78;1-2 11-129.

16. Bilag 5 – Ozon, HPP og CP – kød og kødprodukter

Table 1. Ozone applications to decontaminate meat and meat products.

Sample	Specification	Microbes	Highlights	Reference
Chicken legs	2–10 mg/L for 1 h combined with vacuum packaging (polyamide/polyethylene bags) stored at 4 °C for 16 days.	TVC, *Pseudomonas* spp., LAB, Yeast-molds, & *Enterobacteriaceae*	6-day shelf-life extension compared to vacuum packaging alone (4-day extension). Positively affected odor, texture, and taste retained an acceptable score for 14–16 days.	[29]
Chicken meat (freeze-dried)	0.6 ppm at 4 °C (90% RH) for 10 min.	TAMB, LAB, *E. coli.* & *Salmonella* spp.	1.1 log CFU/g was observed in TAMB and LAB. *E. coli.* and *Salmonella* spp. was not detected. Combination with MAP (20% CO_2, 80% N_2) improved the texture and sensory proprieties.	[30]
Chicken meat (freeze-dried)	0.4–0.7 ppm at 4 °C (90% RH) for 10–120 min.	LAB & TAMB	Reduced 4.77 and 6.8 log CFU/g, respectively. The combined use of ozone and lyophilization would be useful for extending shelf-life to 8 months.	[22]
Chicken breast meat	10×10^{-6} kg $O_3/m^3/h$ for 3 days.	Coliform, aerobic, and anaerobic bacteria	Aerobic: 2.96 log CFU/g (untreated = 5.35 log CFU/g) Anaerobic: 2.18 log CFU/g (untreated = 4.63 log CFU/g) Coliform: 1.74 log CFU/g (untreated = 3.35 log CFU/g)	[31]
Duck breast meat			Aerobic: 2.52 log CFU/g (untreated = 4.11 log CFU/g) Anaerobic: 3.46 log CFU/g (untreated = 3.95 log CFU/g) Coliform: 1.39 (untreated = 3.28)	
Turkey breast meat	1×10^{-2} kg/m^3 at 22 °C (21.6% RH) for 8 h.	TAMB, *Enterobacteriaceae* & yeast-mold	Reduced 2.9, 2.3 and 1.9 log CFU/g, respectively.	[32]
Beef (sliced)	218–286 mg/m^3, 5–20 pulses for 2–40 min with intervals of 30 min.	Heterotrophic microflora & *L. monocytogenes*	Decreased 1.5 log CFU/g heterotrophic counts. Decreased inoculated *L. monocytogenes* counts by more than 1 log CFU/g. Exposure times of more than 10 min negatively affected red color and rancidity.	[33]

O_3: Ozone; TVC: Total viable counts; TAMB: Total aerobic mesophilic bacteria; LAB: Lactic acid bacteria; RH, relative humidity; MAP: Modified atmosphere packaging.

Table 2. HHP applications to decontaminate meat and meat products.

Meat Type	Treatment Conditions	Storage Conditions	Findings	Reference
Chicken fillets	500 MPa for 10 min.	4 and 12 °C	HHP resulted in the reduction of the pathogen population below the detection limit of the enumeration method (0.48 log CFU/g), irrespective of the inoculum. HHP extended the shelf life of chicken fillets by 6 and 2 days, at 4 and 12 °C, respectively.	[44]
Frozen chicken breast	500 MPa for 1 min and 400 MPa for 5 min.	–	HHP showed inactivation of *Salmonella* at 400 MPa for 5 min and 500 MPa for 1 min.	[45]
Ground chicken meat	350 MPa for 10 min + 0.75% carvacrol.		HHP with 0.60% carvacrol treatment resulted in a >5-log pathogen reduction.	[46]
Ground beef	400 MPa for 15 min at 25, 35, and 45 °C.	4 and −20 °C for up to 5 days	At 25 °C, 5 log reduction in *E. coli* O157:H7 was observed further low-temperature storage serves as the hurdle in its survival and recovery after treatment. HHP showed no effect on the chromatic profile of grounded beef.	[47]
Vacuum-packed ground beef	200 and 400 MPa for 5 min at 25 °C.	–	*L. sakei* is good pressure-resistant lactic acid bacteria used in combination with HHP at 400 MPa and is efficient in controlling pathogenic *E. coli* strains.	[48]
Uncooked ground beef patties	300, 400, and 500 MPa for 5 min.	4 °C for 10 days	HHP combine with *Lactobacillus acidophilus* showed less total aerobic count (3.35 log CFU/g) than untreated (6.74 log CFU/g) beef patties with 0.80 log CFU/mL yeast and mold count. The combined treatment showed a delayed decrease in pH value, inhibited lipid oxidation with better color retention and the highest sensory score.	[49]
Beef patty	400 and 600 MPa for 5 min.	Refrigerated storage for 18 h	An amount of 2 and 4 log CF/mL reductions after 400 and 600 MPa in Shiga toxin-producing *E. coli* O157:H7, respectively. Variations in fat concentration of 10 and 20% did not affect. In contrast, 1% NaCl evident more reduction than 2%, indicating bar protective effect of salt.	[50]
Vacuum-pack ripened mutton patties	200 and 400 MPa for 10 min.	4 °C for 28 days	Significant reduction in total plate count after HHP at both levels, with a significant increase in lightness (L*). Redness (a*), yellowness (b*); hardness, gumminess, and chewiness of patties reduced significantly.	[51]
Beef steak	450 MPa, 600 MPa 1, 3, 6, 10, 15 min.	–	HHP have the potential to allow the production of a convenient and safe product by achieving 5 log definition of pasteurization of beef steak inoculated with *E. coli* 0157:H7.	[52]

Table 2. *Cont.*

Meat Type	Treatment Conditions	Storage Conditions	Findings	Reference
Beef slurry	600 MPa for 20 min at 75 °C.	–	Best inactivation of spores of *Clostridium perfringens* in beef slurry was a 2.2 log reduction.	[53]
Beef slurry	600 MPa for 20 min at 75 °C.	–	After HHP, a greater reduction (2.2 log) in *C. perfringens* spores was observed as compared to thermal treatment (no reduction) after 20 min.	[54]
Beef slurry	600 MPa at 70 °C for 20 min.	–	A 4.9 log reduction in *Bacillus cereus* spores after treatment at 70 °C but same temperature thermal processing led to 0.5 log reduction in spore. Increasing HHP temperature from 38 to 70 °C increases the spore inactivation for up to 3 logs.	[55]
Marinated beef (*Longissimus lumborum*)	300, 400, and 600 MPa for 5 min.	Refrigerated storage for 14 days	HHP was proven to provide safe meat along with a sodium reduction in it. Meat marinated with salt and citric acid has no sufficient inactivation of *L. innocua* and *Enterococcus faecium*, while when combine with HHP, a 6 log cycle reduction was observed.	[56]
Beef burgers	300 MPa for 10 min at 9.9 °C and 600 MPa 10 min, 10.2 °C.	–	Mesophilic and psychotropic count remain at the detection limit after HHP at 600 MPa, with no effect on lipid oxidation for at least 6 days.	[57]
Raw meatballs (beef, veal, beef + veal + pork)	400 and 600 MPa for 0 and 18 min.	4 and −12 °C for 18 h	No difference in the extent of inactivation in different species of meat used for meatballs preparation in refrigerated storage (0.9 to 2.9 log CFU/g) as compared to frozen samples (1.0 to 3.0 log CFU/g). A total of 600 MPa requires 1–3 min and 400 MPa requires 9 min for a $\geq$2.0 log CFU/g reduction.	[58]
Emulsified beef sausages	100–400 MPa for 15 min at 10 °C.	–	HHP proved to be an effective technique to produce microbial safe beef sausages (reduce total viable count equivalent to the sausages having higher salt concentration) with lower salt concentration.	[59]
Dry fermented sausages	600 MPa for 3 min.	4 °C for 4 weeks	Inactivation of *E. coli* O157:H7 in dried fermented sausages was observed to be affected by a_w. At $a_w \leq 0.90$, or moisture protein ratio in the range of 1.9–2.3, led to 6.4 log reduction. Further drying reduced to 2.2 log reduction. Recovery of *E. coli* O157:H7 was observed for 1 week of storage but in 2-, 3-, and 4-week storage, no further recovery was observed.	[60]

Table 2. *Cont.*

Meat Type	Treatment Conditions	Storage Conditions	Findings	Reference
Pork cooked sausages	600 MPa for 3 min.	4 and 10 °C for 35 days	Cooking of sausages leads to a >6 log reduction in inoculated *L. monocytogenes*. During storage at 4 °C, no significant growth was observed after HHP. But at 10 °C storage, growth remains below the detection limit up to 21 days after the 4.5 log CFU/mL increase in population was observed. No lactic acid bacterial growth was observed till the end of storage.	[61]
Italian salami	600 MPa for 300 s.	–	HHP related microbial inactivation depicts an inverse relation with a_w. All 20 salami samples showed a 5 log reduction in *Salmonella* after treatment.	[62]
Italian salami	600 MPa for 300 s.	–	An amount of 0.34–4.32 log CFU/g reduction during processing in *L. innocua* was observed which was reduced to 0.48–3.4 log CFU/g after HHP. The efficacy of HHP was associated with a_w and higher pH after acidification, drying and seasoning phase.	[63]
Nitrite-free emulsion-type sausage	0.1, 500 MPa for 12 min + 0, 1, 2% vinegar	4 °C for two weeks followed by at 20 °C for three weeks	HHP (500 MPa; four cycles and each for 3 min) + vinegar (1%) reduced vegetative cells and spores of *C. perfringens* by 4.8 and 2.8 log CFU/g, respectively.	[64].
Traditional Portuguese ready-to-eat meat sausage (*Chouriço de carne*)	300 MPa for 5 min at 10 °C + lactic acid bacteria (Pediococcus acidilactici, HA-6111-2) and its bacteriocin (bacHA-6111-2).	Refregrated storage for 60 days.	The hurdle technology (bacteriocin and pressurization) showed a 0.5 log CFU/g decrease in *L. innocua* cells compared to non-treated cells.	[65]
Dry-cured ham	450 MPa for 10 min and 600 MPa for 5 min.	4 °C for 30 days	The efficacy of HHP against *L. monocytogenes* was reduced by low a_w values. The changes in HHP-surviving bacteria gene transcription patterns were strain-dependent.	[66]
Cooked ham	400 MPa for 10 min at 17 °C + alginate films containing enterocins.	1 or 6 °C for 2 months	Both antimicrobial packaging and pressurization delayed the growth of *L. monocytogenes* levels below the detection limit (day 90) during 6 °C storage.	[67]

HHP: High hydrostatic pressure; a_w: Water activity.

Table 3. CP applications to decontaminate meat and meat products.

Sample	Experimental Conditions	Target Microbes	Remarks	Citation
Chicken breast meat	In-package DBD-CP: 55–80 kV for 3 min, stored at 4 °C for 24 h or 3 days.	Mesophiles or Psychrophiles	Significant decreases in microbial populations after storage of treated sample for 3 days at 4 °C.	[77]
	In-package CP: 80 kV for 180 s at 25 °C and stored at 4 °C.	Mesophiles, Psychrophiles & *Pseudomonas* spp.	High microbial counts in air packed sample (>6 log CFU/g) than in MAP (<4 log CFU/g) stored for 7 days and 14 days (<6 log CFU/g).	[78]
	32 kHz for 10 min + *Crocus sativus* L., *Allium sativum* L., and *Zataria multiflora Boiss*	*E. coli* & *Staph. aureus*	CP with essential oils reached a satisfactory load below 3.5 log CFU/g. Negative effect on odor, flavor, and overall acceptability.	[79]
	In-package DBD-CP: 100 kV for 1–5 min.	Natural microflora	2 log CFU/g reduction within 5 min in Mesophiles, Psychrotrophic & *Enterobacteriaceae*.	[80]
	DBD-CP: 70 kV for 0–300 s, stored at 4 °C for 5 days.	*Psychrophiles Campylobacter jejuni* & *S. typhimurium*	90% reductions in *Psychrophile*; and 0.5, 0.4, and 0.7 log reductions in *psychrophiles*, *Salmonella*, and *Campylobacter*.	[81]
	In-package CP: 60–80 kV for 60–300 s, stored at 4 °C for 5 days.	*Campylobacter* & *Salmonella*	1.0 log reduction in psychrophiles at 60 kV with 35% O_2. Also, 60 kV for 60 s treatment with 35% O_2/60% CO_2/5% N_2 reduces microbes and appearance of meat.	[82]

Table 3. *Cont.*

Sample	Experimental Conditions	Target Microbes	Remarks	Citation
Chicken breast (boiled)	In-package CP: 39 kV for 3.5 min.	*E. coli* O157:H7, *Salmonella*, *L. monocytogenes* & Tulane virus	3.7 log CFU/cube *Salmonella*, 3.9 log 28 CFU/cube *E. coli* O157:H7, 3.5 log CFU/cube *L. monocytogenes*, and 2.2 PFU/cube TV reduction after treatment.	[83]
	In-package DBD-CP: 38.7 kV for 0.3–2.5 min	*Salmonella*	Whey protein coating increased treatment efficacy. An increase in initial inoculum concentration from 3.8 to 5.7 log CFU/sample lead to an increase in D-value increased from 0.2 to 1.3 min with1.7 log CFU/sample (highest) *Salmonella* reduction.	[84]
RTE chicken breast cubes	In package CP: 24 kV for 3 min, stored at 4 °C for 21 days.	Mesophilic aerobic bacteria, *Salmonella*, & Tulane virus	0.7, 1.4 and 1.1 log PFU/cube reduction in mesophilic aerobic bacteria, *Salmonella*, and Tulane virus, respectively.	[85]
Chicken breast patties (ground)	DBD-CP: 70 kV for 180 s at 22 °C, packaged in operating gas: 65% O_2, 30% CO_2.	Total plate count	0.9 log reduction after 5-day storage as compared to non-CP treated samples. Rosemary extract prevents lipids oxidation and inhibits microbial growth in CP-processed meat under refrigerated conditions.	[86]
Chicken skin & breast fillet	Plasma jet: Feed gases (argon or air) for exposure times (30–180 s), distances from plasma jet nozzle to sample surface (5–12 mm).	*Campylobacter jejuni*	0.78 to 2.55 and 0.65 to 1.42 log CFU/cm^2 reductions were observed using argon or air as feed gases, respectively. Argon as a feed gas for a longer time (≥120 s) resulted in the highest reductions.	[76]
Chicken meat	DBD-CP + Paraacetic Acid (PPA 100–200 ppm) 3.5 kHz, 0–30 kV, 0–200 W. for 1–6 min, 2 mm distance.	*S. typhimurium*	2.3 to 5.3 log CFU/cm^2 reductions with combined treatment in contrast to PAA or CP treatments alone. 4.7 and 5.3 log CFU/cm^2 was the highest reduction obtained after PAA + CP and CP + PAA, respectively.	[73]
Beef	6 kV and 20 kHz for 30 s–10 min.	*E. coli*	0.9 and 1.82 log CFU/cm^2 reduction after 2- and 5 min treatments, respectively.	[75]
Pastırma (a dry-cured beef product)	Oxygen (100%), argon (100%) and two oxygen/argon mixtures (25%O_2/75%Ar and 50%O_2/50%Ar) for 180 and 300 s.	*L. Monocytogenes*, *Staph. aureus*, total mesophilic aerobic bacteria & yeast–mold	Maximum 0.85 log CFU/cm^2 and 0.83 log CFU/cm^2 reduction in *S. aureus* and *L. monocytogenes* counts, respectively. 1.41- and 1.66-log CFU/cm^2 reduction in total mesophilic aerobic bacteria and yeast–mold counts, respectively.	[28]
Pork loin	DBD-CP: 80 kV for 60–180 s.	Total Aerobic bacteria	53% reduction in total aerobic bacteria showed a significant effect on O_2 concentration (60%) and time (180 s).	[87]
Pork (fresh & frozen)	Plasma jet: Air 20 kV, 58 kHz, 1.5 A for 0–120 s	*L. monocytogenes* & *E. coli* O157: H7	1.5 log and >1.0 log reduction in *E. coli* O157: H7 and *Listeria monocytogenes*, respectively.	[74]

Table 3. *Cont.*

Sample	Experimental Conditions	Target Microbes	Remarks	Citation
Ham	2 and 10 kHz, 6.4 or 10 kV for 10–20 min at 22 °C.	*S. typhimurium* & *L. monocytogenes*	1.14 log and 1.02 log reduction in *S. Typhimurium* and *L. monocytogenes* log after 20 min, respectively. CP combined with cold storage for 7–14 days at 8 °C packed under sealed high nitrogen gas flush (70% N_2, 30% CO_2) effectively inactivating *S. Typhimurium* (1.84 log) and *L. monocytogenes* (2.55 log).	[88]
RTE ham	In-package DBD-CP: 3.5 kHz, 0–30 kV for 23 °C and stored at 4 °C for 18 h.	*L. monocytogenes*	2 log (CFU/cm^2) reduction after CP combined with MAP (20% O_2 + 40% N_2 + 40% CO_2) and after 7 days storage at 4 °C cell counts reduced below the detection limit (>6 log reduction).	[89]
	In-package DBD-CP: 3.5 kHz, 0–28 kV for 180 s, and stored for 6 and 24 h at 4 °C.	*L. innocua*	At 4 °C, 1.75 and 1.51 log CFU/cm^2 reduction on 1% and 3% NaCl ham surface, respectively. At 23 °C, 1.78 and 1.43 log CFU/cm^2 reduction, respectively.	[90]
RTE mortadella-type sausage	18 kV, 12.5 kHz for 0–120 s, 6 mm distance. Samples were sealed under high nitrogen gas flush (70% N_2, 30% CO_2) and stored at 4 °C for 1–21 days.	*E. coli*, *L. monocytogenes* & *S. enterica* serovar *Typhimurium*	The maximum inactivation for *Salmonella* was 0.3 logs. After 120 s CP and storage over 21 days counts for *Listeria* as well as *E. coli* were lower compared to a 30 s treatment (6.58 to 6.25 log and 5.63 to 5 log CFU/g, respectively).	[91]

CP: Cold plasma; DBD: dielectric barrier discharge; RH: relative humidity; MAP: modified atmosphere packaging; PAW: Plasma activated water; RTE: Ready-to-eat.

Tabel 1,2 og 3 i bilag 5 er kopieret fra: Roobab, U., Chacha, J.S., Abida, A., Rashid, S., Madni, G.M., Lorenzo, J.M., Zeng, X.-A. & R.M. Aadil (2022) Emerging Trends for Nonthermal Decontamination of Raw and Processed Meat: Ozonation, High-Hydrostatic Pressure and Cold Plasma. Foods 11, 2173.

Table 2
Single and combined high power ultrasound applications and microbial reductions in the wash-water decontamination process of meats.

Product	Microorganisms	Reduction (log CFU)	Application	Parameters	References
Broiler drumsticks	Aerobic bacteria	0.–0.8/cm^2	Ultrasound + 1% Lactic acid	47 kHz, 200 W 25–40 °C, 15–30 min.	Sams and Feria (1991)
Broiler breast skin	*Salmonella typhimurium*	1–1.5/ml 2.4–3.9/ml	Ultrasound + peptone Ultrasound + 0.5 ppm chlorine	20 kHz, 15–30 min.	Lillard (1993)
Broiler wing skin	Aerobic bacteria	1.8/ml	Ultrasound Distilled water	40 kHz, 2 W/cm^2, 20 °C, 6 min	Stasiak et al. (2007)
	Salmonella	3.6/ml	Ultrasound + 1% Lactic acid		
Broiler carcass	*Campylobacter*	2.5/carcass	Ultrasound + steam	Sonosteam technique	Boysen and Rosenquist (2009)
Pork meat	*B. thermosphacta* *L. monocytogenes* *C. jejuni* *C. maltaromaticum*	1.2/ml 1/ml 1/ml 0.8/ml	Ultrasound + marination in red wine	25 kHz–300 W, and 1 MHz–150 W, 10 min, 12 °C	Birk and Knøchel (2009)
Broiler breast	*E. coli* *Salmonella typhimurium*	3.3/ml 2.5/ml	Ultrasound + marination (12 °C, 10 min)	Not reported	Smith (2011)
Chicken wing skin	Gram-negative bacteria	1.0/cm^2 ≤4.0/cm^2	Ultrasound + water Ultrasound + 1% Lactic acid	40 kHz, 2.5 W/cm^2	Kordowska-Wiater and Stasiak (2011)
Pork meat	*E. coli* *S. typhimurium* *Y. enterocolitica*	2.5/cm^2 2/cm^2 2.1/cm^2	Ultrasound + steam (130 °C, 3.5–5 atm)	30–40 kHz, 2 sec.	Morild et al. (2011)
Chicken drumstick	*Campylobacter*, enterobacteriaceae, total viable count	No significant reduction	Ultrasound	20 W/L, 16 min, ≤28 °C	Haughton et al. (2012)
Chicken breast	Psychrophilic bacteria	0.2/g	Ultrasound	20 kHz, 5 min	Piñon et al. (2012)

Tabel 2 i bilag 6 er kopieret fra: Turantas, F., Kilic, G. B. & B. Kilic (2015) Ultrasound in the meat industry: General applications and decontamination efficiency. International Journal of Food Microbiology 198, pp. 59-69.

Table 3
Microbial reduction levels for meat, fish, derived products and cheese after pulsed light processing.

Food product	Microorganism treated	Operation conditions	Reduction (log_{10} CFU/g)	Reference
Stainless steel in contact with meat	*Listeria monocytogenes* *Escherichia coli*	Total fluence (J/cm^2): 3; Pulse width (μs): 300; Discharge voltage (V): 3000; Distance from the lamp (cm): 10	6.5	Rajkovic et al. (2010)
Tuna *carpaccio* Beef *carpaccio*	*Listeria monocytogenes* *Escherichia coli* *Salmonella Typhimurium* *Vibrio parahaemolyticus*	Total fluence (J/cm^2): 11.9; Peak power (J/cm^2/pulse): 0.175; Pulse width (μs): 250	Beef carpaccio: 0.9 Tuna carpaccio: 0.7 Beef carpaccio: 1.2 Beef carpaccio: 1.0 Tuna carpaccio: 1.0	Hierro et al. (2012)
Shrimp, salmon, flatfish	*Listeria monocytogenes*	Frequency (Hz): 5; Number of pulse: 6900; Total fluence (J/cm^2): 12.1; Peak power (J/cm^2/pulse): 0.00175; Exposure time (s): 1380	Shrimp: 2.4 Salmon: 2.1 Flatfish: 1.9	Cheigh et al. (2013)
Dry cured meat products (salchichón and loins)	*Listeria monocytogenes* *Salmonella enterica serovar Typhimurium*	Total fluence (J/cm^2): 11.9; Peak power (J/cm^2/pulse): 0.7; Pulse width (μs): 250	Salchichón: 1.81 Loins: 1.61 Salchichón: 1.48 Loins: 1.73	Ganan, Hierro, Hospital, Barroso, and Fernández (2013)
Raw pork roast, roast pork and raw salmon	*Aerobic flora* *Pseudomonas fluorescens*	Frequency (Hz): 1; Total fluence (J/cm^2): 30; Distance from the lamp (cm): 3	Raw pork roast: 0.96 Roast pork: 0.99 Raw Salmon: 0.7 1.0–1.5	Nicorescu, Nguyen, Chevalier, and Orange (2014)
White cheddar cheese	*Listeria innocua* *Pseudomonas fluorescens* *Escherichia coli*	Total fluence (J/cm^2): 3.1; Pulse width (μs): 360; Pulse-repetition-rate (pulses/s): 3;	3.0 3.0 5.0	Proulx et al. (2015)
Seafood Isolates	*Listeria monocytogenes* *Listeria innocua*	Pulse energy (J/cm^2): 0.316; Peak power (J/cm^2/pulse): 0.053 Pulse width (μs): 325; Distance from the lamp (cm): 11	2.4 5.4	Lasagabaster and de Marañón (2017)
Sliced fermented salami	*Listeria monocytogenes* *Escherichia coli* *Salmonella Typhimurium* *Staphylococcus aureus*	Total fluence (J/cm^2): 3; Number of pulses: 1; Pulse width (μs): 300; Discharge voltage (V): 3000; Distance from the lamp (cm): 10	2.24 2.29 2.25 2.12	Rajkovic et al. (2017)
Pork skin	*Salmonella typhimurium* *Yersinia enterocolitica*	Total fluence (J/cm^2): 19.11; Distance from the lamp (cm): 8.3; Pulse width (μs): 300; Treatment time: 30 s	2.97 4.2	Koch et al. (2019)
Pork loin	*Salmonella typhimurium* *Yersinia enterocolitica*	Total fluence (J/cm^2): 0.52–19.11; Distance from the lamp (cm): 8.3–13.4; Pulse width (μs): 300; Treatment time: 1–30 s	0.4–1.6 0.4–1.7	

Tabel 3 i bilag 7 er kopieret fra: Mahendran, R., Ramanan, K. R., Barba, F.J., Lorenzo, J.M., López-Fernández, O., Munekata, P.E.S., Roohinejad, S., Sant'Ana, A.S. & B.K. Tiwari (2019) Recent advances in the application of pulsed light processing for improving food safety and increasing shelf life. Trends in Food Science & Technology 88:67-79, https://doi.org/10.1016/j.tifs.2019.03.010.

Table 17.2 Combined Effect of Non-Thermal Technologies and Conventional Preservation Techniques

	Conventional Hurdles		
Non-Thermal Technology	**Heat**	**pH**	**Antimicrobials**
High hydrostatic pressure	+++	±	++/+++
Pulsed electric field	+++	±	+++
Ultrasonication	+++	---	++/+++
Continuous Ultraviolet	+++	---	++
Pulsed light technology	+++	---	++/+++
Ozonation	+++	---	++
Irradiation	---	---	
Electron beam technology	---	---	++/+++,*

Table 17.4 Combined Preservation Effect of Different Non-thermal Technologies

	Non-thermal hurdles								
Non-thermal technology	**High hydrostatic pressure**	**Pulsed electric field**	**Ultrasonication**	**Continuous Ultraviolet**	**Pulsed light technology**	**Ozonation**	**Irradiation**	**Supercritical CO_2**	**Microfiltration**
High hydrostatic pressure	___	+++	+++	NL	NL	++	+++	++	+++
Pulsed electric field	+++	___	+++	+++	+++	++/+++	NL	+++	+++
Ultrasonication	+++	+++	___	+++	++/+++	+++	+++	+++	
Continuous Ultraviolet	NL	+++	+++	___	NL	+++	NL	NL	NL
Ozonation	++	++/+++	+++	+++	NL	___	NL	NL	NL
Irradiation	+++		+++	NL	NL	NL	___	NL	NL

+ No change in effect
++ Additive effect
+++ Synergistic effect
^ Antagonistic effect
± Mixed results
* Adverse effects on Sensory properties
NL No literature available

Tabeller i Bilag 8, kopieret fra: Sethi, S., Anurag, R.K., Yogesh Kumar, Y. & O. P. Chauhan (2019) in Chauhan, O.P. (Ed.): Non-thermal Processing of Foods (1st ed.). CRC Press. https://doi.org/10.1201/b22017.

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