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Review

The application of carbon monoxide in meat packaging needs to be re-evaluated within the EU: An overview



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ABSTRACT

Carbon monoxide (CO) has many value-added benefits in meat packaging due to its colour stabilising effects and enhancement of meat quality attributes. The regulation of CO within meat packaging varies worldwide and remains a topical and controversial issue. CO is prohibited in the EU for use in meat packaging mainly due to fears it may mask spoilage therefore misleading consumers. The issue of consumer acceptance of CO was not considered. This article reviews the most pertinent literature to assess if the problems associated with the prohibition have been addressed. Applying CO pretreatments prior to vacuum packaging enhances colour while allowing discolouration to occur by the use-by-date, thereby addressing concerns about safety. Recent work showing European consumer acceptance of CO in meat packaging demonstrates its future potential within the EU. The information provided may support framing future policies intended to assure consumer protection, safety, choice and interest. Re-evaluation of permitting CO as a packaging gas within the EU may be warranted.

1. Introduction

Maintaining an attractive colour during retail display remains a challenge to the meat industry. Consumers use “colour” as a reliance quality cue to determine whether a product is safe and fresh to consume (Greibitus, Jensen, & Roosen, 2013) and colour determines the consumer's perceived quality of meat (Issanchou, 1996). The bright red colour of meat is associated with product freshness and wholesomeness (Kropf, 1980). In recent years, consumers seek more information about the food they consume, whether it is safe to eat, the quality of meat, authenticity, origin and production method or system (Woolfe, 2012). Consumers have a negative attitude towards additives and there has been a growing trend towards healthier and naturally sourced clean label ingredients (Hung, de Kok, & Verbeke, 2016; Martins, Roriz, Morales, Barros, & Ferreira, 2016). The meat industry faces the challenge of finding an acceptable clean label ingredient for meat packaging which enhances meat colour. Consumers also have a negative perception of additives even though approved additives have been proven safe for human consumption and many have been derived from natural sources and are not synthetic.

Carbon monoxide (CO) has a history of application within the meat industry as a colour enhancer due to its colour stabilising effects coupled with its antioxidant abilities. The use of CO as a packaging gas has many benefits including increased colour stability, shelf-life extension due to

microbial inhibition properties, enhanced flavour, reduced protein oxidation and lipid oxidation, improved tenderness and prevention of premature browning (El-Badawi, Cain, Samuels, & Anglemeier, 1964; Gee & Brown, 1978; Carpenter, Cornforth, & Whittier, 2001; Jayasingh, Cornforth, Carpenter, & Whittier, 2001; Krause, Sebranek, Rust, & Honeyman, 2003; Hunt et al., 2004; Seyfert, Hunt, Mancini, Kropf, & Stroda, 2004; John et al., 2004, 2005; Mancini, Hunt, Hachmeister, Kropf, & Johnson, 2005; Cornforth & Hunt, 2008). The application of low concentrations of CO (0.4%) for fresh muscle cuts and ground meat prior to vacuum packaging can maintain product freshness and wholesomeness, assist flexibility during distribution and prevent meat shrinkage (FDA, 2012). However, there is inconsistency worldwide in the regulation of its use within the meat industry and the use of CO is currently receiving attention among researchers and industry. Differing regulations globally can be a non-tariff barrier to trade limiting the possibilities for exports between countries (Greibitus, Jensen, & Roosen, 2013).

Globally, the European Food Safety Authority (EFSA), European Commission (EC) and Food and Drug Administration (FDA) are important regulatory authorities. Their responsibilities include adherence to consumer safety and protection, and ensuring quality products which promote human health. EFSA are the foundation of the European safety system and provide scientific advice to the European Commission (EC) prior to policy making. However, the regulations of these

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authorities differ regarding the acceptance of certain additives, more specifically, the acceptance of CO as a packaging gas.

In the USA, CO was first regulated as a secondary packaging gas in 2002 (FDA, 2002). In 2004, the FDA recognised a concentration of 0.4% CO as generally recognised as safe (GRAS) and permitted CO as a primary packaging gas in case-ready packaging systems within the USA (FDA, 2004). However, packages are required to be labelled with “use or freeze by” date of 35 days for intact steaks or roasts and 28 days for mincemeat (FDA, 2004). In addition, packages are required to state “colour is not an accurate indicator of freshness”. This is to ensure consumer safety and to avoid misleading consumers about product freshness as CO can maintain an acceptable colour beyond the spoilage shelf life and consumers generally use colour as a determinant of quality (FDA, 2012). However, since this labelling regulation was enacted, some retailers and some packers in the USA more commonly apply CO as a secondary packaging gas in a master bag system as labelling is not required in this case. Additionally consumers take very little CO home with them using this packaging form.

New Zealand and Australia also regulate low concentrations of CO in centralised packaging systems and it is considered a processing aid (Federal Register of Legislative Instruments, 2014). Similarly, Canada also allows the application of 0.4% CO as a secondary packaging gas (USDA-FSIS, 2016). Norway applied low concentrations of CO within meat packaging systems for nearly 20 years. However, the regulation of its use ended in 2004 due to the adoption of EU regulations to increase trade with the EU.

In the EU packaging gases are considered as additives and require an “E number” (Walsh & Kerry, 2002). EU legislation (Directive No 89/107/EEC and Directive No 95/2/EC), which applies to additives other than colours and sweeteners and refers specifically to “packaging gases” states that “packaging gases are gases other than air, introduced into a container before, during or after the placing of a foodstuff in that container”. Modified atmosphere packaged foods require labelling which states “Packaged in a protective atmosphere” and E numbers should be displayed on the label e.g. E290 for CO₂ and E948 for O₂ (European Parliament and Council Directive, 1995; Sorheim & Nissen, 2000). In order for CO to be approved as an additive within the EU the following criteria must be met according to Directive No 89/107/EEC:

- “there can be demonstrated a reasonable technological need and the purpose cannot be achieved by other means which are economically and technologically practicable,
- they present no hazard to the health of consumer at the level of use proposed, so far as can be judged on the scientific evidence available,
- they do not mislead the consumer.”

CO however has not yet been approved as a packaging gas. In 2004, the European Parliament prohibited the use of CO in meat packaging systems. This may have been due to concerns about CO masking the microbial spoilage of meat which can mislead consumers about product freshness and be a consumer safety concern (European Commission, 2001). Meat safety is considered by consumers a prerequisite (Van Wezemael, Verbeke, Kügler, de Barcellos, & Grunert, 2010). Another reason may have been the hazardous potential of the gas which consumers may perceive negatively (Cornforth & Hunt, 2008). This was contradicted by the European Scientific Committee which stated that low levels of 0.3% - 0.5% CO mixed with carbon dioxide (CO₂) and nitrogen (N₂) used as a modified atmosphere gas for meat stored at 4 °C presented no health threat (European Commission, 2001). Additionally, Sorheim, Aune, and Nesbakken (1997) published a report on the toxicity of CO which concluded that the application of low concentrations of CO to meat packaging systems were consumer friendly and no toxic effects were evident. An exposure to a low concentration of CO (50 ppm or less) in the air for 8 h is considered to be safe (Sebranek & Houser, 2006).

Several EU countries have made on-going efforts to permit the

application of CO within the EU under carefully controlled and regulated conditions; however it may take some time to effect a change (Sorheim et al., 2006). Another important issue with the prohibition is that consumers' preferences were not considered (Greibitus, Jensen, & Roosen, 2013). A consumer's personal knowledge and media exposure can affect willingness to pay (WTP) (Greibitus, Jensen, Roosen, & Sebranek, 2013). Concerns of CO being a potentially hazardous gas can negatively influence consumers' acceptance of CO as a packaging gas as well as concerns for workers' safety. Therefore consumer acceptance of CO as a packaging technology needs to be considered.

The aim of this article was to review the most recent literature on the benefits and consumer safety issues related to the use of CO in meat packaging and consumer acceptance of CO in meat packaging systems. The purpose of this was to assess if the previous issues related to the prohibition of CO within the EU have been addressed and warrant the re-evaluation of whether CO should be permitted as a packaging gas within the EU. This information could further assist framing future policies including protection, safety, choice and interest (Greibitus, Jensen, & Roosen, 2013). If CO was to be permitted as a packaging gas within the EU, the European processors would be able to export CO pretreated meat to countries where CO is permitted. Furthermore, application of CO within the EU could improve profitability and exports for meat producers and retailers as well as meet consumer quality demands as a value added technology (Greibitus, Jensen, Roosen, et al., 2013).

2. Current meat packaging issues

Meat packaging innovations can play a pivotal role in meeting the goal of a sustainable future and ensuring food security. It has been estimated that > 20% of the 263 million tonnes of meat produced globally is lost or wasted, which equates to 75 million bovine raised for no reason (FAO, 2016; Saucier, 2016). The global population is estimated to increase from 7.4 billion to 9.7 billion by 2050 thereby requiring higher meat supplies. Meat consumption is estimated to continue to increase by 17.5% to 356 million tonnes from 2013 to 2023 (OECD-FAO, 2014). Therefore, with an increasing meat consumption and meat supply required, waste needs to be minimised to contribute to global food security and a sustainable future. A major contributing factor towards meat waste at retail level, mainly in Europe, North America and Industrialised Asia, is consumer discrimination towards discoloured meat products (FAO, 2016) which consumers perceive as unwholesome (Faustman & Cassens, 1990). However, colour has no effect on taste (Carpenter et al., 2001). In order to reduce food waste and support increased consumer demand and expectation of high quality value-added meat, packaging technology innovations are required. Packaging has a direct influence on the colour and quality of meat (Bernuésa, Olaizolab, & Corcoran, 2003). There is a growing demand for more tender meat products. Therefore, packaging technologies which provide both desirable colour and increased tenderness are necessary. However, meat is considered a perishable food due to its biological activity. This can have a negative effect on the colour and is a challenge for the meat industry. Colour is the foremost quality cue judged during consumer purchasing as other quality attributes including tenderness, flavour and juiciness cannot be assessed prior to consumption. Therefore, the meat industry has strived over many decades to prolong the storage life, prevent deterioration and maintain the colour as well as enhancing the eating quality attributes of fresh meat.

At present, the meat industry generally employs a two-stage packaging system where primals are aged in vacuum packs (VP) (“wet aged”) before being sliced and transferred to retail VP, vacuum skin packs (VSP) or modified atmosphere packs (MAP). Many of these packaging technologies have been extensively reviewed therefore only the key issues are highlighted in this review.

High oxygen (O₂) MAP is a commonly applied second-stage fresh red meat packaging technology. The inclusion of O₂, usually at 75–80%, results in the formation of a deep layer of oxymyoglobin, which produces the desirable red colour, while the CO₂ is included for its bacteriostatic effect (McMillin, 2008). Unfortunately, extended exposure to O₂ can cause lipid and protein oxidation thereby limiting the shelf-life and leading to reduced juiciness, decreased tenderness and the development of off-flavours (Tørngren, 2003; Clausen, 2004; Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010). During transportation and distribution, the bulky MAP packs are more susceptible to leakages which can contribute to revenue losses (Siegel, 2011).

VP is an anoxic technology and is primarily used within the meat sector for the prolonged storage of fresh primals or subprimals, for distribution to foreign markets by shipment (Egan, Eustace, & Shay, 1988; Mansur, 1997). VP prevents lipid oxidation, delays microbial spoilage and is the most cost effective, commonly applied method of ageing used to increase tenderness (Eilert, 2005; Obuz, Akkaya, Gök, & Dikeman, 2014). Consumers consider tenderness as the most essential palatability attribute which determines the overall eating experience (Grobbel, Dikeman, Hunt, & Milliken, 2008) and can determine consumer repurchases (Hur, Jin, Park, Jung, & Lyu, 2013). The increased consumer demand for meat tenderness has highlighted the benefits of applying VP within the meat industry. However, the major issue for the success of VP is consumer acceptance of VP meat products which are presented with a dark purple (deoxymyoglobin) appearance, which is perceived as unattractive (Carpenter et al., 2001). Despite a great effort by one of the largest commercial meat companies and retailers in the US, the marketing of individual VP meat cuts was not successful (Lawrence & Kropf, 2014). College students assisted the marketing in an attempt to educate consumers that VP meat is perfectly acceptable, however while quality was perfectly acceptable, colour did not meet consumer expectations (Lawrence & Kropf, 2014). However, while this may be the case for US consumers a study carried out by Van Wezemael, Ueland, and Verbeke (2011) reported that European consumers found VP was the most accepted packaging technology (73%) while MAP was the second most accepted (53%). Another disadvantage of VP is purge held in the folds of the vacuum package which often leads to increased microbial growth and is less appealing to consumers (Li, Lindahl, Zamaratskaia, & Lundstrom, 2012).

VSP is a more recent packaging technology that has been designed to prevent package purge loss while maintaining many of the benefits of VP. VSP involves the meat product being placed on a tray which is then covered by an upper and lower layer of packaging film where the upper layer is heated which enables a tight seal over the surface of meat product using vacuum (Lagerstedt, Ahnstrom, & Lundstrom, 2011; Taylor, Down, & Shaw, 1990; Vazquez et al., 2004). This consequently prevents package purge, increases product appeal and prevents off-odours (Taylor et al., 1990). VSP is increasing in the marketplace in recent years particularly within the EU due to the demand for more tender meat and prolonged shelf-life. Other benefits of VSP include facilitating smaller meat cuts and better portion control which con-

tribute to preventing obesity and cater for single-person households, providing convenience and preventing food waste (Bord Bia, 2011). The O₂ deficit environment of VSP has similar limitations to that of VP in that the meat has a dark purple colour due to deoxymyoglobin (Lagerstedt, 2011).

A possible solution to overcome the unattractive colour of VP or VSP may be to expose the meat to a gas mixture containing CO as a pretreatment prior to VP or VSP packaging. This will induce a desirable red colour that will last throughout the display period, coupled with allowing ageing to occur within the package. Thus, the negative quality issues related to high O₂ MAP packaging can be avoided. However, there is no literature which states a commercial application of this technique within the meat sector (Robertson, 2010). Furthermore this technology could only be implemented if CO was permitted within the EU as a packaging gas.

3. Role of CO in meat colour

CO may be described as an odourless, tasteless and colourless gas which is produced by the incomplete combustion of carbon containing materials (Sorheim, Aune, & Nesbakken, 1997; Zhang, Yu, Xiao, Wang, & Tian, 2013). CO can readily bind to myoglobin to form carboxymyoglobin, which presents a bright cherry red colour similar to oxymyoglobin (El-Badawi et al., 1964). CO is considered highly stable and myoglobin has a 28–51 times greater affinity for CO than for O₂ (De Santos, Rojas, Lockhorn, & Brewer, 2007). Thus carboxymyoglobin is more resistant to oxidation in comparison to oxymyoglobin and is widely known for its antioxidant properties. Additionally, carboxymyoglobin has a visual spectrum which is similar to that of oxymyoglobin (Gee & Brown, 1978).

3.1. Sources of CO

CO is naturally synthesised within the human body caused by the breakdown of haemoproteins. A concentration of 1.2–1.5% carboxyhaemoglobin (HbCO) is endogenous in non-smokers, while in smokers it is 3–4% (European Commission, 2001) (Table 2). The European Commission (2001) reported that inhalation of CO-MAP headspace gas containing 0.3% - 0.5% CO would have no significant effect on the carboxyhaemoglobin (HbCO) in the blood in comparison to other sources of inhalation of CO. The report also stated that the amount of CO present in fresh meat packaged in low concentrations of CO-MAP is similar to that of the endogenous CO. It is also important to note that during cooking a considerable amount (~85%) of CO which is bound to carboxymyoglobin and carboxyhaemoglobin of the packaged meat is lost. Human exposure to CO (mg/m³ or ppm) and the amount of carboxyhaemoglobin (CO Hb %) concentrations in the blood are presented in (Tables 1 & 2).

Table 1

Human exposure to CO (mg/m³ or ppm).

Extracted from: (Sorheim, Aune, & Nesbakken, 1997; WHO, 2000; European Commission, 2001; (EEA (European Environment Agency), 2012; EPA, 2017).

Source	Human exposure to CO (mg/m ³ or ppm)	References
Natural background levels (e.g. oxidation of methane in troposphere, decay of chlorophyll, carbon containing materials)	0.01–0.9 mg/m ³ ; 0.06–0.14 mg/m ³ (0.05–0.12 ppm)	(European Commission, 2001; WHO, 2000)
Petrol-fuelled or faulty propane-fuelled ice resurfacers used at ice-hockey games from an average of 7 arenas (average ice-hockey game period 1.5–2 h)	40–46 mg/m ³ (35–40 ppm)	(WHO, 2000)
Large European cities (8 h exposure)	< 20 mg/m ³ (17 ppm)	(WHO, 2000)
Environmental tobacco smoke in dwellings, offices, vehicles and restaurants (8 h exposure)	23–46 mg/m ³ (17 ppm)	(WHO, 2000)
Homes with gas appliances	60–115 mg/m ³ (53–100 ppm)	(WHO, 2000)
Maximum recommended exposure	9 ppm of CO (8 h exposure); 35 ppm (1 h exposure); 10 mg/m ³ (8 h daily mean)	EEA (European Environment Agency), 2012; EPA, 2017)

Table 2

Carboxyhaemoglobin (CO Hb %) concentrations in the blood.

Extracted from: (Aunan et al., 1992; Sorheim, Aune, & Nesbakken, 1997; WHO, 2000; European Commission, 2001; Sorheim, Nissen, Aune, & Nesbakken, 2001; Bjørlykke, Kvamme, Slinde, & Sorheim, 2012).

Source	Concentration in human blood (CO Hb %)	References
Production of CO exogenous in the human body due to the breakdown of haemoproteins	0.5%; 0.4–0.7%	(European Commission, 2001; Sorheim, Aune, & Nesbakken, 1997; WHO, 2000; Bjørlykke et al., 2012)
Pregnant woman	0.7–2.5%	(WHO, 2000)
Foetuses of non-smoker mothers	0.4–2.6%	(WHO, 2000)
Hypermetabolism patients caused by haemolytic anaemia & certain drugs	4–6%	(WHO, 2000)
Non-smokers (exogenous combined with environmental factors e.g. pollutants)	1.2–1.5%	(European Commission, 2001; Sorheim, Aune, & Nesbakken, 1997; Sorheim et al., 2001)
Smokers/cigarette smoke	3–4%	(Aunan et al., 1992; European Commission, 2001; Sorheim, Aune, & Nesbakken, 1997; Sorheim et al., 2001)
Healthy adults with no adverse health effects	< 5%	(Sorheim, Aune, & Nesbakken, 1997)
Vulnerable individuals should not exceed	~2%	(Sorheim, Aune, & Nesbakken, 1997; European Commission, 2001)

4. Historical perspective of the use CO

CO has a long history of applications within the food industry, including, meat, fish, vegetable and fruit processing (Cornforth & Hunt, 2008). Over a century ago, a patent was approved for the application of CO₂/CO gaseous mixtures to extend the shelf-life of meat (Church, 1994). El-Badawi et al. (1964) was one of the first studies to report that colour stability in fresh beef could be increased to a period of 15 days with the addition of 2% CO to air. Similarly, Clark, Lentz, and Roth (1976) reported that a lower concentration of 1% CO mixed with 99% N₂ had similar effects with increased colour stability and prolonged odour shelf-life in beef samples. Further benefits of CO were demonstrated by Gee and Brown (1978), who reported that atmospheres of 1% CO, 50% CO₂ and 49% air increased the microbial shelf-life and colour stability by 4.5 days in minced meat stored at 2 °C (Gee & Brown, 1978). From 1985 to 2004, the Norwegian meat sector used CO concentrations of 0.3–0.5% in MAP and case-ready packaging systems of beef, pork and lamb (Sorheim, Erlandsen, Nissen, Lea, & Hoyem, 1997; Sorheim, Nissen, & Nesbakken, 1999). During this period 60% of all retail meat marketed in Norway was packaged using low concentrations of CO (Sorheim, 2006). Additionally, the USA have applied CO within vegetable processing since the 1970's to prolong the shelf-life of iceberg lettuce during distribution (Mermelstein, 1977; Kader, 1983) and it is currently applied in centralised meat packaging systems in the USA.

5. Benefits of CO in meat packaging

Many researchers have reported numerous benefits of applying CO to meat packaging systems under refrigerated storage conditions, including increased colour stability, prolonged shelf-life due to microbial inhibition, decreased protein oxidation, enhanced flavour, improved tenderness in anaerobic packaging systems, absence of bone darkening and prevention of premature browning (El-Badawi et al., 1964; Gee & Brown, 1978; Carpenter et al., 2001; Jayasingh et al., 2001; Krause et al., 2003; Seyfert et al., 2004; Hunt et al., 2004; John et al., 2004, 2005; Mancini et al., 2005; Cornforth & Hunt, 2008). Krause et al. (2003) reported that CO-MAP with mixtures of 0.5% CO, 70% and 29.5% N₂ significantly enhanced the colour and sensory attributes of pork chops in comparison to overwrap, VP and MAP. Additionally, lipid oxidation was reduced when compared to overwrap packaging. While numerous studies have reported the effects of CO on extended colour stability, additional benefits of the application of CO have also been reported. Woodruff and Silliker (1985), reported a concentration of 10% CO can penetrate 0.63–0.94 cm beneath the surface of meat, forming a bright stable red carboxymyoglobin layer while inhibiting microbial growth, further preventing odour and slime

by-products. Additionally, Clark et al. (1976) showed increasing CO concentrations with the balance gas being N₂ on beef rump steaks inhibited the growth of psychotropic bacteria which also had a positive effect on increased odour shelf-life. This result was due to CO having the ability to increase the lag phase and reduce the log phase. CO has also been reported to prevent the growth of bacteria, yeasts and moulds (Mansur, 1997). Pathogenic bacteria such as *Escherichia coli* O157 (*E. coli* O157) were reported to be inhibited in ground beef samples enclosed in CO-MAP gas mixtures of 0.4% CO, 60% CO₂, 39.6% N₂ (Nissen, Alvseike, Bredholt, Holck, & Nesbakken, 2000). Brooks et al. (2008) concluded similar results with CO-MAP systems with a 0.4% CO concentration where pathogenic *salmonella* and *E. coli* O157 were reduced in comparison to packaging systems without CO. Other pathogens reported to be inhibited by low concentration of CO and high CO₂ include *Listeria monocytogenes* and *Yersinia enterocolitica* (European Commission, 2001). CO has also been reported to inhibit food spoilage bacteria including *Brochothrix thermosphacta*, *Pseudomonas*, *Pseudomonas fluorescens* and *Achromobacter* (Gee & Brown, 1978; Sorheim et al., 1999).

Furthermore, John et al. (2005) reported that beef steaks packaged in 0.4% CO-MAP or in VP can prevent premature browning and reduce oxidation and rancidity. Ultra low O₂ atmospheres with a concentration of 0.4% CO have also resulted in increased tenderness when compared to high-O₂ MAP (Grobbe et al., 2008). Additionally, Liu et al. (2014) reported that 0.4% CO-MAP packaging systems can maintain higher metmyoglobin reducing activity (MRA), which is linked to increased colour stability, compared to high-O₂ MAP.

6. Application of CO pretreatment in fresh meat

A possible response to the consumer resistance to the purple colour of VP beef is the application of CO as a pretreatment prior to packaging. Applying CO pretreatments prior to VP has previously been reported by numerous researchers (Clark et al., 1976; Lentz, 1979; Rozbeh, Kalchayanand, Field, Johnson, & Ray, 1993; Brewer et al., 1994; Jayasingh et al., 2001; Sagarnaga, 2006; Aspé, Roeckel, Martí, & Jiménez, 2008; O'Connor & Allen, 2011; Sakowska, Guzek, Glabska, & Wierzbicka, 2016; Sakowska, Guzek, Sun, & Wierzbicka, 2016; Sakowska, Guzek, & Wierzbicka, 2016; Van Rooyen, Allen, Crawley, & O'Connor, 2017; Van Rooyen, Allen, Gallgaher, & O'Connor, 2016) (Table 3). Brewer et al. (1994) reported that exposing beef to CO prior to VP can maintain the red colour in the VP. Krause et al. (2003), suggested that the pretreatment of fresh meat prior to VP would promote the desirable cherry red pigment for 21 days during centralised packaging and distribution, due to the stability of the carboxymyoglobin layer which maintains the red appearance in the absence of CO-MAP. A report by Humphreys (1996) stated that approximately 97% of beef in the USA

Table 3
Published studies assessing the effect of CO pretreatment on meat.

Sample	Pretreatment exposure time	Gas concentration %					Packaging conditions	Storage conditions	Main effects	References
		CO	CO ₂	N ₂	Air	O ₃				
Beef rump muscle	2–16 h				1		PVC film (air permeable bag)	0, 5, 10 °C	CO pretreatment did not increase colour shelf-life or odour due to storage in O ₂ permeable packs	(Clark et al., 1976)
Beef short hip muscle	3 h	100					Vacuum packaged	Frozen storage, –7, –18, –29 °C	Extended colour shelf-life of 90 days and samples unaffected by light during frozen storage.	(Lentz, 1979)
Beef <i>semitendinosus</i> steaks	0.5 h	100					Vacuum packaged	3 °C to 8 weeks	Increased colour stability for 8 weeks	(Rozbeh et al., 1993)
Beef <i>semitendinosus</i> steaks	1 h	10		90			Vacuum packaged	3 °C to 8 weeks	Extended colour shelf-life for 4 weeks	(Rozbeh et al., 1993)
Beef Ribeye, top round and eye of round muscles	0.5 h	100					Vacuum packaged	4 ± 2 °C for 42 days	Colour stability for 6 weeks and discoloured by end of storage with no differences between CO-treated and untreated steaks. Antimicrobial effect for APC & LAB with 1 log reduction after 8 weeks storage, 2 log reduction for psychrotrophic counts.	(Brewer, Wu, Field, & Ray, 1994)
Beef short loin	24 h	5	60	35			Vacuum packaged	2 °C for 5 weeks	Colour stability for 5 weeks. Most preferred treatment due to safety of handling lower concentrations of CO. Microbiological growth exceeded 10 ⁶ cfu/cm ² . Ideal packaging system should provide 21 days display which was achieved.	Jayasingh et al., 2001
Beef short loin	1 h	100					Vacuum packaged	2 °C for 6 weeks	Colour stability for 6 weeks. Microbiological growth exceeded 10 ⁶ cfu/cm ²	(Jayasingh et al., 2001)
Pork <i>Longissimus thoracis et lumborum</i> (LTL)	1 h	1	99				Vacuum packaged	5 ± 0.5 °C for 20 days	Increased colour shelf-life and had the highest consumer acceptance after 24 h storage from sensory visual tests (best treatment). CO did not have any antimicrobial effect.	(Vianna, Gomide, & Vanetti, 2005)
Pork <i>Longissimus thoracis et lumborum</i> (LTL)	1 h	100					Vacuum packaged	5 ± 0.5 °C for 20 days	Increased colour stability. Most accepted after 20 days storage. Microbial load exceeded 10 ⁷ CFU/g and off-odour was detected.	(Vianna et al., 2005)
Pork & Beef <i>Semimembranosus muscles</i> for fermented meat model systems (batter used to prepare salami sausages) & salami sausages	5 day	1		99			Vacuum packaged	3 °C for 3 months followed by 3 °C display for 32 days	CO could be used as alternative colourant to nitrite to increase colour stability. Vacuum packaging was effective in preventing discoloration in sausages compared to air storage	(Sorheim et al., 2006)
Pork & Beef <i>Semimembranosus muscles</i> for cooked model meat systems (batter to prepare hotdogs) & hotdogs	1 day	1		99			Vacuum packaged	Frozen at –20 °C for 3 months followed by 3 °C display for 32 days	CO could be used as alternative colourant to nitrite to increase colour stability. CO-pretreatment applied to cooked batters during the last stage of the batter chopping was optimum to increase colour stability.	(Sorheim et al., 2006)
Beef striploin, inside round and top sirloin	30 min	100					Vacuum packaged	4 °C for 21 d display	CO-pre-treatment increased colour stability, while not masking microbial spoilage. CO-VAC was more tender and juicy.	(Sagamaga, 2006)
Beef Chops (bone-in) <i>Longissimus thoracis et lumborum</i> (LTL)	24 h	5		95			Vacuum packaged	2 ± 0 °C for 12 weeks	Colour stability and for 11 weeks and shelf-life of 7 weeks, pH, water-holding capacity, purge and rancidity not affected	(Aspé et al., 2008)
Beef <i>Longissimus thoracis et lumborum</i> (LTL)	30 min – 72 h	5	60	35			Vacuum packaged	4 °C for 21 d display	Colour stability reduced over storage (21 days). CO did not mask microbial spoilage.	(O'Connor & Allen, 2011)
Beef <i>Longissimus thoracis et lumborum</i> (LTL)	48 h	0.1–0.5	30	69.5–69.9			Vacuum packaged	2 °C for 21 d display	Polish consumers accepted and found the 0.3% and 0.5% CO pretreatment prior to vacuum packaging to be the desirable and attractive due to enhanced	(Sakowska, Guzek, & Wierzbicka, 2016)

(continued on next page)

Table 3 (continued)

Sample	Pretreatment exposure time	Gas concentration %					Packaging conditions	Storage conditions	Main effects	References
		CO	CO ₂	N ₂	Air	O ₃				
Beef <i>Longissimus thoracis et lumborum</i> (LTL)	48 h	0.5	30	69.5			Vacuum packaged	1 ± 2 °C for 21 d storage in the dark	appearance compared to untreated vacuum packaged steaks. The red colour was maintained for 21 days for 0.3% and 0.5% CO pretreatment for 48 h. Polish consumers preferred cherry/dark red and purple colour of 0.5% CO pretreated steaks after 21 days compared to CO-MAP and untreated vacuum packaged steaks. CO-pretreatment had no effect on, pH, WBSF while CO-Vac had higher microbial counts than CO-MAP possibly due to fabrication. CO-pretreatment and vacuum packaged steaks had higher drip loss than CO-MAP. Increased CO concentration increased colour stability and CO penetration depth. Applying 0.5% CO pretreatment for 48 h prior to vacuum packaging may enhance colour while avoid persistent pinking in cooked samples. The 5% CO pretreatment for 5 h was the optimum exposure time to enhance colour and did not mask meat spoilage as discoloration occurred by use-by-date, irrespective of display temperature. K/S ratios, further confirms that CO pretreatment did not mask spoilage and could be more sensitive than colour parameters at monitoring discoloration. Exposure to 5% CO pretreatment did not have any negative effect on meat quality attributes including cooking loss, tenderness, purge loss, pH, while mild temperature abuse (6 °C) increased purge loss and decreased pH as expected. CO pretreatment had significantly higher colour than untreated vacuum packaged steaks (control). Drip loss, microbiological safety, metmyoglobin, TBARS, total volatile basic nitrogen and pH were lower than the control. Ozone combine CO can enhance meat quality. Increased colour stability. Colour reduction over storage (28 days) with 5 h treatment being optimum exposure time to discolor by use-by-date and colour did not mask spoilage. No negative effect on microbiological safety, tenderness, cooking loss and lipid oxidation.	(Sakowska, Guzek, Sun, & Wierzbicka, 2016)
Beef <i>M. longissimus lumborum</i> (LL)	48 h	0.1–0.5	30	69.5–69.9			Vacuum packaged	2 °C for 21 days in storage the dark	Increased CO concentration increased colour stability and CO penetration depth. Applying 0.5% CO pretreatment for 48 h prior to vacuum packaging may enhance colour while avoid persistent pinking in cooked samples. The 5% CO pretreatment for 5 h was the optimum exposure time to enhance colour and did not mask meat spoilage as discoloration occurred by use-by-date, irrespective of display temperature. K/S ratios, further confirms that CO pretreatment did not mask spoilage and could be more sensitive than colour parameters at monitoring discoloration. Exposure to 5% CO pretreatment did not have any negative effect on meat quality attributes including cooking loss, tenderness, purge loss, pH, while mild temperature abuse (6 °C) increased purge loss and decreased pH as expected. CO pretreatment had significantly higher colour than untreated vacuum packaged steaks (control). Drip loss, microbiological safety, metmyoglobin, TBARS, total volatile basic nitrogen and pH were lower than the control. Ozone combine CO can enhance meat quality. Increased colour stability. Colour reduction over storage (28 days) with 5 h treatment being optimum exposure time to discolor by use-by-date and colour did not mask spoilage. No negative effect on microbiological safety, tenderness, cooking loss and lipid oxidation.	(Sakowska, Guzek, Glabska, & Wierzbicka, 2016)
Beef <i>Longissimus thoracis et lumborum</i> (LTL)	3–7 h	5	60	35			Vacuum packaged	2 °C (good practice) or 6 °C (mild temperature abuse) for 28 day display	Increased CO concentration increased colour stability and CO penetration depth. Applying 0.5% CO pretreatment for 48 h prior to vacuum packaging may enhance colour while avoid persistent pinking in cooked samples. The 5% CO pretreatment for 5 h was the optimum exposure time to enhance colour and did not mask meat spoilage as discoloration occurred by use-by-date, irrespective of display temperature. K/S ratios, further confirms that CO pretreatment did not mask spoilage and could be more sensitive than colour parameters at monitoring discoloration. Exposure to 5% CO pretreatment did not have any negative effect on meat quality attributes including cooking loss, tenderness, purge loss, pH, while mild temperature abuse (6 °C) increased purge loss and decreased pH as expected. CO pretreatment had significantly higher colour than untreated vacuum packaged steaks (control). Drip loss, microbiological safety, metmyoglobin, TBARS, total volatile basic nitrogen and pH were lower than the control. Ozone combine CO can enhance meat quality. Increased colour stability. Colour reduction over storage (28 days) with 5 h treatment being optimum exposure time to discolor by use-by-date and colour did not mask spoilage. No negative effect on microbiological safety, tenderness, cooking loss and lipid oxidation.	(Van Rooyen, Allen, Gallagher, & O'Connor, 2016)
Beef <i>Longissimus lumborum</i> and <i>psosas</i> major	1.5 h	90–100				2–10	Vacuum packaged	0 °C for 46 day storage	Increased CO concentration increased colour stability and CO penetration depth. Applying 0.5% CO pretreatment for 48 h prior to vacuum packaging may enhance colour while avoid persistent pinking in cooked samples. The 5% CO pretreatment for 5 h was the optimum exposure time to enhance colour and did not mask meat spoilage as discoloration occurred by use-by-date, irrespective of display temperature. K/S ratios, further confirms that CO pretreatment did not mask spoilage and could be more sensitive than colour parameters at monitoring discoloration. Exposure to 5% CO pretreatment did not have any negative effect on meat quality attributes including cooking loss, tenderness, purge loss, pH, while mild temperature abuse (6 °C) increased purge loss and decreased pH as expected. CO pretreatment had significantly higher colour than untreated vacuum packaged steaks (control). Drip loss, microbiological safety, metmyoglobin, TBARS, total volatile basic nitrogen and pH were lower than the control. Ozone combine CO can enhance meat quality. Increased colour stability. Colour reduction over storage (28 days) with 5 h treatment being optimum exposure time to discolor by use-by-date and colour did not mask spoilage. No negative effect on microbiological safety, tenderness, cooking loss and lipid oxidation.	(Lyu, Shen, Ding, & Ma, 2016)
Beef <i>Longissimus thoracis et lumborum</i> (LTL)	1–24 h	5	60	35			Vacuum packaged	2 °C for 28 day display	Increased CO concentration increased colour stability and CO penetration depth. Applying 0.5% CO pretreatment for 48 h prior to vacuum packaging may enhance colour while avoid persistent pinking in cooked samples. The 5% CO pretreatment for 5 h was the optimum exposure time to enhance colour and did not mask meat spoilage as discoloration occurred by use-by-date, irrespective of display temperature. K/S ratios, further confirms that CO pretreatment did not mask spoilage and could be more sensitive than colour parameters at monitoring discoloration. Exposure to 5% CO pretreatment did not have any negative effect on meat quality attributes including cooking loss, tenderness, purge loss, pH, while mild temperature abuse (6 °C) increased purge loss and decreased pH as expected. CO pretreatment had significantly higher colour than untreated vacuum packaged steaks (control). Drip loss, microbiological safety, metmyoglobin, TBARS, total volatile basic nitrogen and pH were lower than the control. Ozone combine CO can enhance meat quality. Increased colour stability. Colour reduction over storage (28 days) with 5 h treatment being optimum exposure time to discolor by use-by-date and colour did not mask spoilage. No negative effect on microbiological safety, tenderness, cooking loss and lipid oxidation.	(Van Rooyen et al., 2017)

meat market experiences fabrication or VP at some stage during processing or distribution. Therefore implementing CO pretreatments prior to packaging would not be unfamiliar.

CO pretreatments have long since been employed. Clark et al. (1976) reported improved colour stability and decreased odour for beef rump muscles stored in 0.5–10% CO-MAP atmospheres at 0, 5 and 10 °C. However, in the same study pre-treatments of 99% CO and 1% air followed by air storage gave no further colour or odour decrease. Later work did show a positive effect from exposing beef samples to 100% CO for 3 h before VP as colour shelf-life was increased (Lentz, 1979). Furthermore, Rozbeh et al. (1993) pretreated beef *semitendinosus* steaks with gas mixtures of 10% CO + 90% N₂ for 60 min and 100% CO for 30 min at 3 °C before VP. A stable red colour was maintained during 4 weeks storage for muscles pre-treated with 10% CO, while beef treated with 100% CO obtained a colour shelf-life of 8 weeks (Rozbeh et al., 1993). A study carried out by Brewer et al. (1994) reported colour stability effects and microbial inhibition for beef steaks due to the 100% CO pretreatment for 30 min prior to vacuum packaging at refrigerated temperatures. A study carried out by Brewer et al. (1994) reported colour stability effects and microbial inhibition for beef rib-eye and round steaks due to the 100% CO pretreatment for 30 min prior to vacuum packaging at refrigerated temperatures. CO pretreatment significantly enhanced redness up to 6 weeks compared to the untreated control. Additionally, an extended shelf-life for CO pretreated steaks due to microbial inhibition was observed as after 8 weeks of storage there was a 1 log reduction for aerobic plate counts and lactic acid bacteria (LAB) counts in comparison to an untreated control and a 2 log reduction for psychotropic bacteria counts. Similarly, Jayasingh et al. (2001) investigated the colour stability of beef steaks exposed to pretreatments of 100% CO for 1 h and 5% CO for 24 h, prior to vacuum packaging. The 5% CO pretreatments prolonged the colour shelf-life for 5 weeks whereas the 100% CO pretreatment maintained a colour shelf-life of 6 weeks. The objective of the experiment was to achieve a colour stability of 21 days (maturation (7 days), retail display (7 days) and consumer use by date (7 days)) with the lowest CO concentration. This was achieved using a concentration of 5% CO. CO pre-treatments before vacuum packaging for retail would greatly assist distribution within the meat industry. Aspé et al. (2008) analysed the effect of CO pretreatment and film properties on the quality of VP beef chops. They applied a pretreatment of 5% CO with 95% N₂ to beef chops for 24 h at 2 °C before VP in thermo-contractile packs or non-heat-contractile packs. The microbial shelf-life was extended to 11 weeks in both types of VP, however heat-contractile packs reduced purge loss and improved colour stability compared to non-heat contractile packages. Furthermore pH, water holding capacity, purge loss, and rancidity were not influenced by CO pretreatment. A study carried out by Sagarnaga (2006) compared three different packaging technologies; MAP (80% O₂/20% CO₂), CO/VAC 100% CO pretreatment for 30 min prior to VP and CO/MAP 100% CO pretreatment for 30 min before packaging in MAP (30% CO₂/70% N₂). The study showed that CO/VAC increased colour stability and extended shelf-life without masking microbial spoilage and improved overall acceptability compared to CO/MAP and MAP (Sagarnaga, 2006). Additionally, CO-VAC was more tender and juicy than other treatments. The benefits of CO pretreatments prior to VP compared to CO-MAP include a more compact pack size thereby reducing storage and lowering costs during transportation thus increasing profitability for industry (Sakowska, Guzek, Sun, et al., 2016).

Although CO has many advantages, concerns have been raised in the past that CO may mask the microbial spoilage of meat (Kropf, 1980). The high stability of carboxymyoglobin can lead consumers to falsely perceive the freshness of meat as colour is used as an indicator of freshness. Eilert (2005) disagreed and reported that CO does not mask spoilage and can reduce flavour oxidation and odour. Sorheim (2006) reported that varying the meat exposure period to CO during pretreatment could be used to regulate the colour stability during display without masking microbial spoilage. A preliminary study carried out by

O'Connor and Allen (2011) applied pretreatment regimes to beef *Longissimus thoracis et lumborum* (LTL) steaks to find a regime that would allow the formation of metmyoglobin to occur by the use-by date. The study was designed to combine the advantages of VP with the cherry-red colour of steaks in CO-MAP packaging while ensuring that discolouration occurred by the end of the shelf life to address consumers concerns about spoilage being masked. The study included variations in the exposure times of 5% CO from 30 min to 72 h. An initial bright cherry red colour reduced over a storage period of 21 days. Following this, Van Rooyen et al. (2017) determined the optimum exposure time with a 5% CO concentration to be 5 h as this allowed discolouration to occur by the end of a 28-day display period (2 °C). The 5% CO pre-treatment for 5 h had no negative effect on microbiological safety, cooking loss, tenderness and lipid oxidation at the end of storage. Therefore these results address the previous issues associated with the ban of CO as microbial spoilage was not masked if the correct combination of exposure time and CO concentration is applied. The CO exposure time was greatly reduced compared to previous studies which applied a 5% CO pretreatment for 24 h (Jayasingh et al., 2001; Aspé et al., 2008), thus potentially improving efficiency and reducing process time.

The European Commission (2001) also raised a valid concern that if CO meat products are stored under inappropriate conditions, for example increased temperature which can occur during mishandling or transportation; the presence of CO may mask visual evidence of spoilage. Therefore, Hunt et al. (2004) reported that 0.4% CO-MAP did not mask meat spoilage in ground beef, loineye, inside round and tenderloin after storage under inappropriate storage conditions of mild temperature abuse (6 °C) as microbial spoilage was not accelerated. Van Rooyen et al. (2016), found that mild temperature abuse (6 °C) did not alter the conclusion that a 5% CO pretreatment for 5 h did not mask spoilage as discolouration occurred by use-by-date, irrespective of display temperature. The only effect of the higher temperature on quality attributes was an increased purge loss and decreased pH.

Another important issue to address if CO is to be approved within the EU is persistent pinking (carboxymyoglobin layer being retained after cooking), which has been an on-going problem in the US especially for meat which has been exposed to CO-MAP. However, the application of CO pretreatments prior to vacuum packaging may overcome this issue as CO is not present in the pack during storage. The very short exposure time to CO may reduce or eliminate the formation of persistent pinking after cooking. Recently, Sakowska, Guzek, Glabska, and Wierzbicka (2016) reported that reducing the CO-MAP concentration to 0.1% or using a 0.5% CO pretreatment exposure time of 48 h prior to vacuum packaging can prevent the persistent pinking in cooked beef striploin steaks. However, it is important to note that carboxymyoglobin formation is proportional to gas concentration and exposure time.

Also, further research is needed on various muscle types as each muscle type has specific physiological properties which influence colour stability and post-mortem biochemical changes (Seyfert, Mancini, Hunt, Tang, & Faustman, 2007). Therefore on-going, research by this group (unpublished results) is investigating the CO pretreatment of steaks from different muscles.

7. Consumer perception and acceptance of CO

Consumers' attitudes, perception, responses and acceptance regarding novel packaging technologies can also vary globally (Greibitus, Jensen, & Roosen, 2013). The prohibition of the application of CO in MAP within the EU did not consider consumer preferences when the ban was put in place as the Commission presumed consumers' purchase intent of CO meat packaging systems would not be of interest (Greibitus, Jensen, Roosen, & Sebranek, 2013). Sensitivity to information, personal knowledge and media exposure of CO may negatively influence consumer acceptance of CO meat packaging (Greibitus,

Jensen, & Roosen, 2013; Grebitus, Jensen, Roosen, & Sebranek, 2013). Therefore a choice study was carried out by Grebitus, Jensen, and Roosen (2013) on ground beef which evaluated German and US consumer acceptance and WTP of packaging technologies such as MAP and CO-MAP which included labelling on the packages, informing the consumers of improved meat quality attributes due to the packaging technology type. US consumers preferred prolonged shelf-life and clear information about the packaging technology. However, WTP for CO-MAP decreased for US consumers when labelling information was included about the role of CO as a colour stabiliser but did not affect WTP of German consumers. Overall, the results indicated that German consumers would prefer to have an option of food packaging technologies applied with modified atmosphere gases such as CO as consumers were attracted to the enhanced meat colour. Additionally, German consumers were willing to pay €3.75 more for a desirable cherry red appearance in ground beef while US consumers would only pay an additional €0.69. However, consumers in both countries had increased preference towards enhanced cherry red colour. This result agrees with work carried out by Carpenter et al. (2001) and Killinger, Calkins, Umberger, Feuz, and Eskridge (2004) who both concluded that consumers have a preference for the bright cherry red colour.

Similarly two recent consumer studies were carried out on Polish consumers (Sakowska, Guzek, Sun, & Wierzbicka, 2016; Sakowska, Guzek, & Wierzbicka, 2016) to evaluate whether Polish consumers would accept CO in meat packaging systems. The first study by Sakowska, Guzek, and Wierzbicka (2016) evaluated consumer preferences towards CO pretreatments (0.1%, 0.3% and 0.5%) of beef striploin steaks exposed for 48 h prior to VP compared to untreated vacuum packaged steaks. Consumers had a preference and increased desire to purchase steaks packaged after 0.3% and 0.5% CO pretreatments as they had the most attractive cherry red colour. Consumers did not accept untreated vacuum packaged beef steaks as they were considered the least attractive and desirable. Following this, these authors carried out another study (Sakowska, Guzek, Sun, & Wierzbicka, 2016) whereby Polish consumer acceptance and preference of CO-MAP (0.5%) was compared to CO pretreatments (0.5%) prior to VP. In agreement with the previous study (Sakowska, Guzek, and Wierzbicka, 2016), Polish consumers preferred and had the highest likelihood to purchase for CO pretreatments (0.5%) in comparison to CO-MAP.

Therefore, the results from these studies in two European countries (Germany and Poland) show promise for the future potential application of CO within the EU, despite the current EU prohibition of MAP with CO. Consumer acceptance and WTP of CO in meat packaging within the EU can provide beneficial information to assist future marketing and product positioning in the market and increase profitability for meat producers and retailers due to enhanced value-added and meat quality attributes. Moreover this information can assist in the development of future policy making which focuses on the protection of consumer choice and interest (Grebitus, Jensen, & Roosen, 2013).

8. Conclusion

CO as a pretreatment applied prior to VP or VSP may play an important role in assisting packaging innovations to overcome some of the challenges the meat industry faces. It demonstrates future potential as a value added meat packaging technology in terms of maintaining an acceptable colour, providing prolonged storage, meeting consumer demand for increased tenderness as well as preventing negative issues associated with other packaging technologies. The colour stabilising abilities of CO may reduce global food waste due to meat discoloration. As a result this may further assist the goal of a sustainable future, ensuring food security and increase profitability for the meat industry. Allowing the ageing process to take place within the VP or VSP pack as opposed to carcass or primal ageing will decrease energy usage, storage facilities and distribution costs.

This article has reviewed some of the most recent studies of CO in meat packaging systems in order to address the issues associated with the prohibition within the EU. The criteria that must be met for CO to be permitted as an additive within the EU.

(Directive No 89/107/EEC) have been satisfactorily addressed therefore a reconsideration by the EU Commission is justified. CO is widely reported as a colour enhancer for a perishable food product such as meat, therefore providing a technological need. CO has presented no health threat at the level of use proposed as a primary packaging gas in MAP (0.3%–0.5%) for meat stored at 4 °C according to the European Scientific Committee (European Commission, 2001). Additionally low concentrations of CO to meat packaging systems have been reported to be consumer friendly and have no toxic effects (Sorheim, Aune, et al., 1997; Sorheim & Nissen, 2000). CO has previously been reported to mask meat spoilage and this was the primary concern raised for the prohibition as this may mislead consumers. However, Van Rooyen et al. (2017) demonstrated that the application of the 5% CO pretreatment for 5 h prior to vacuum packaging achieves enhanced colour, while allowing discoloration to occur by the end of a 28-day display period (2 °C), so as to not mask spoilage. This ensures consumers have a reliable visual indication of freshness and addresses previous concerns about consumer safety as consumers are not misled by an attractive colour being retained beyond the microbiological spoilage life. However, it is important to note that the correct combination of CO gas concentration and exposure time needs to be determined for the desired use-by date and possibly be varied for different cuts. Recent European consumer acceptance studies from Germany and Poland, demonstrate promising future potential of the application of CO within the EU.

This review could provide useful information to guide the framing of future policies intended to support consumer protection, safety, choice and interest (Grebitus, Jensen, & Roosen, 2013). Additionally, this article may educate consumers of the benefits of CO and resolve any misconception or negative media influence of CO as a packaging gas so that consumers may make an informed choice. In addition, if CO was permitted as a packaging gas within the EU European processors would be able to export CO pretreated meat to countries where CO is permitted, potentially increasing exports and profits. The information provided here may be used to make a case for the re-evaluation of CO as a permitted packaging gas within the EU.

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