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# 谷物和油料中甲酸乙酯和硫化羰本底水平评估（中英文）

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**摘　要：**甲酸乙酯和硫化羰是潜在的储藏物熏蒸剂，广泛存在于大气、植物和土壤中。它们在新收获的谷物和储存的小麦、大麦、燕麦和油菜籽中的本底含量因商品、温度、含水量和储存期而异，其中甲酸乙酯含量为 0.5~2.0 mg/kg，硫化羰含量为 0.02~1.0 mg/kg。两种成分含量在储存后的前 4~5 个月呈上升趋势，然后下降，在温度高于 20 ℃，谷物和油菜籽含水量分别大于 9.5%和 5%时变化最明显。这些本底含量研究对开发甲酸乙酯和硫化羰作为储藏物熏蒸剂时制定最大残留限量（MRL）和市场接受度值有重要参考价值。

**关键词：**甲酸乙酯；硫化羰；熏蒸剂；熏蒸剂本底水平；谷物；油料
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# Evaluation of Background Levels of Ethyl Formate and Carbonyl Sulfide in Grains and Oilseeds (Chinese and English versions)

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**Abstract:** Ethyl formate and carbonyl sulfide are potential stored product fumigants. They are present naturally in the atmosphere, plants and soil. Natural levels of ethyl formate and carbonyl sulfide in newly harvested grain and stored wheat, barley, oats and canola are varied with the commodity, temperature, moisture content and the period of storage. The values ranged from 0.5~2.0 mg/kg for ethyl formate and 0.02~1.0 mg/kg for carbonyl sulfide. Ethyl formate and carbonyl sulfide were naturally present in grains at

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本专栏背景及第一作者、通讯作者、译者介绍详见 PC8-15，本文英文原文详见 P24-P29

harvest, increased during the first 4~5 months of storage, and then began to decline, particularly at grain temperatures greater than 20 ℃ and moisture contents of grain and canola greater than 9.5% and 5%. This information on natural levels is relevant to the establishment of Maximum Residue Limits (MRL) and to market acceptance of ethyl formate and carbonyl sulfide as fumigants for stored products.

**Key words:** ethyl formate; carbonyl sulfide; fumigant; background level of fumigant; grains; oilseeds

甲酸乙酯（EtF）和硫化羰（COS）均具有作为控制储藏物中病虫害熏蒸剂的潜力[1-8]。

甲酸乙酯用于干果[6,9-10]和其他农产品[11-12]熏蒸有着悠久的历史。甲酸乙酯职业安全与健康阈限值（TLV）为 100 mg/m$^3$，即工人在每个班次的工作时间中可以接触且不会产生不利影响的浓度。与其他熏蒸剂相比，甲酸乙酯阈限值高说明其更安全，如磷化氢（$PH_3$）的阈限值为 0.3 mg/m$^3$，甲基溴（MeBr）的为 1 mg/m$^3$。Desmarchelier 等[13]重新评估了甲酸乙酯作为甲基溴的替代品熏蒸储藏物。最近研究发现甲酸乙酯作为熏蒸剂可用于稻谷[14]、大麦[15]和板栗[16]。此外，还对熏蒸后柑橘[17]和蓝莓[18]等水果的品质进行了研究。甲酸乙酯作为熏蒸剂已成功用于控制包装食品、饮料和干果等普通货物中的害虫，且不会影响食品质量[6]。甲酸乙酯是一种天然存在的挥发性物质，存在于空气、牛奶、奶酪、小麦、啤酒和许多新鲜产品中[19]。它会迅速水解为乙醇和甲酸，这两种物质也是生物源化合物[20]。甲酸乙酯通常被认为是安全的，是一种用作调味剂的食品添加剂[21]，尚无证据表明它对公众有害[22]。Desmarchelier[19]评估了一系列产品中的甲酸乙酯本底水平，如蔬菜、储粮和动物产品。认为它是一种天然存在的杀虫化合物，有可能替代对环境有害的合成杀虫熏蒸剂[18]。例如，甲酸乙酯作为一种天然挥发性物质存在于以下产品中：牛奶 0.15 mg/kg[23]、奶酪 1.3 mg/kg[24]、小麦 0.3 mg/kg 和小麦胚芽 0.7 mg/kg[13]、大麦 0.2~1.0 mg/kg[19]和大麦产品如啤酒 0.9~10 mg/kg[25]。

硫化羰（COS）被认为是潜在替代甲基溴（MeBr）的熏蒸剂之一[26-27]。作为甲基溴和磷化氢的替代产品，COS 对磷化氢抗性害虫赤拟谷盗有显著防治效果，而对稻米品质没有显著影响[5]。Desmarchelier[19]审查了一系列产品（如蔬菜、储藏谷物和动物产品）中的 EtF 天然水平。它是一种天然存在的杀虫化合物，有可能替代对环境有害的熏蒸剂[18]。与甲酸乙酯相同，自然界存在的物质，如海洋、土壤、火山和沼泽均可检测到硫化羰[1,28-29]。因此，硫化羰自然存在于大气、水、土壤、植物、食品原料和加工食品中，包括谷物和油籽。谷物和油籽中的硫化羰本底水平为 0.02~ 0.07 mg/kg[13,30-31]。

澳大利亚农药和兽药管理局（APVMA）对甲酸乙酯熏蒸后的储藏物的最大残留限量（MRL）未作要求，因为认为熏蒸残留限量与这些储藏物的本底水平一样，即没有毒理学意义[32]，但目前没有系统的研究证据来支持这一假设。关于温度、含水量、品种和储存期等因素对储藏物中甲酸乙酯和硫化羰本底水平影响的信息很少。为评估甲酸乙酯和硫化羰作为谷物熏蒸剂可行性，需要研究有关储藏物中这些熏蒸剂的本底水平，为制定其中的最大残留限量（MRL）提供依据，从而应用。因此，根据现有文献综述，对粮油产品中的甲酸乙酯本底水平及其影响因素，如储存期、温度和含水量等，开展系统研究具有重要的意义。

本研究中，我们评估了温度、含水量、商品和储存期四个因素对澳大利亚小麦、大麦、燕麦和油菜籽中甲酸乙酯和硫化羰本底水平的影响。选择这几种谷物的原因是，小麦是澳大利亚的第一大谷物，大麦是第二大谷物，其品质对酿酒业尤为重要。燕麦脂质含量为 5.5%~6.5%，是小麦、大麦或黑麦的 2~3 倍，纤维含量为 11%~12%，是大多数谷物的 2~4 倍[33]。油菜籽是澳大利亚的主要油料。

## 1 材料与方法

### 1.1 样品准备

选择澳大利亚四种代表性谷物：小麦、燕麦、大麦和油菜籽进行评价。为避免引入其他污染物，

采用人工收割模式。每个样品取 1.5 kg，放入 2 L 密封罐中，用蒸馏水调节，分别放入(15±1)、(25±2)和(35±2) ℃三个不同温度下平衡 2 周，获得不同含水量的样品，按照国际标准（ISO 712—2015《谷物和谷物制品水分含量的测定》）测定含水量（湿基）。其中，小麦的含水量分别为 11.9%、12.9%和 13.9%；大麦的含水量分别为 12.1%、13.0%和 14.9%；燕麦的含水量分别为 10.8%、12.5%、13.4%；油菜籽的含水量分别为 5.1%、6.7%和 7.9%。每个含水量的谷物分成三份，分别装入 2 L 的广口瓶中并密封，在(15±1)、(25±2)和(35±2) ℃下储藏，每 9 个月取样检测甲酸乙酯和硫化羰含量。

### 1.2　试剂与仪器

硫化羰压缩气体 1 瓶：通过 BOC（澳大利亚）从 Matheson 气体公司（USA）购买；甲酸乙酯：Tech Ajax（Sydney, Australia）；丙酮：Biolab Scientific（澳大利亚维州 Clayton）；其他化学品：BDH AnalaR（英国），所用试剂均为分析纯。

在 250 mL 锥形烧瓶中进行提取，配有磨玻璃接头（Crown Scientific，N.S.W.，Australia，Cat. No.FE250/3）；半孔隔膜的快接塞子（Alltech Associates，P/N 6526）；配备 FPD 检测器的 GC6AM 岛津气相色谱：Shimadzu Seisakusho, Kyoto, Japan；1 m×3 mm（内径）HayeSep Q 的玻璃填充柱：Alltech Associates，Cat. No. 2801；配备FID检测器的Varian Star 3600 CX气相色谱：Varian Associates, lnc., USA；30 m×0.53 mm（内径）DB FFAP 大口径毛细管柱：J&W 125-3212。

### 1.3　硫化羰和甲酸乙酯天然含量的测定

硫化羰采用 GC6AM 岛津气相色谱进行测定，通过 1 m×3 mm（内径）HayeSep Q 的玻璃填充柱进行分离，柱温为 140 ℃，载气流量（$N_2$）为 40 mL/min，压力为 0.8 psi。甲酸乙酯采用 Varian Star 3600 CX 气相色谱进行测定，通过 30 m×0.53 mm（内径）DB FFAP 大口径毛细管柱进行分离，柱温为 70 ℃。

参照 Desmarchelier 等[13]、Le 和 Ren（个人交流）的方法，采用顶空固相微萃取和溶剂浸提法分析硫化羰和甲酸乙酯的本底水平。对于硫化羰，在 250 mL 密封烧瓶的 50 g 样品中加入 50 mL 酸化丙酮水溶液（$H_3PO_4$+水+丙酮，1∶2.2∶12.5，v/v/v）浸提 10 h。对于甲酸乙酯，在 250 mL 密封烧瓶的 50 g 样品中加入 70 mL 硝酸铵（70%，w/v）溶液浸提 24 h。抽取 50 μL 顶空气体进行气相色谱，采用加标法定期校准峰面积，根据峰面积计算硫化羰或甲酸乙酯的含量，图中记录数据为样本的平均值。

### 1.4　气体标准品和加标样本的制备

利用 5 个 1 L 的玻璃烧瓶制备不同浓度梯度的硫化羰或甲酸乙酯。混匀后，使用稀释气体制备加标样本和气体标准品。向装有商品和提取溶液的密封烧瓶中注入一定体积的稀释气体，设定重复组。对于硫化羰，气相色谱进样前，需要对溶剂进行洗脱或蒸发，因此两次进样之间的间隔时间最短为 10 min。

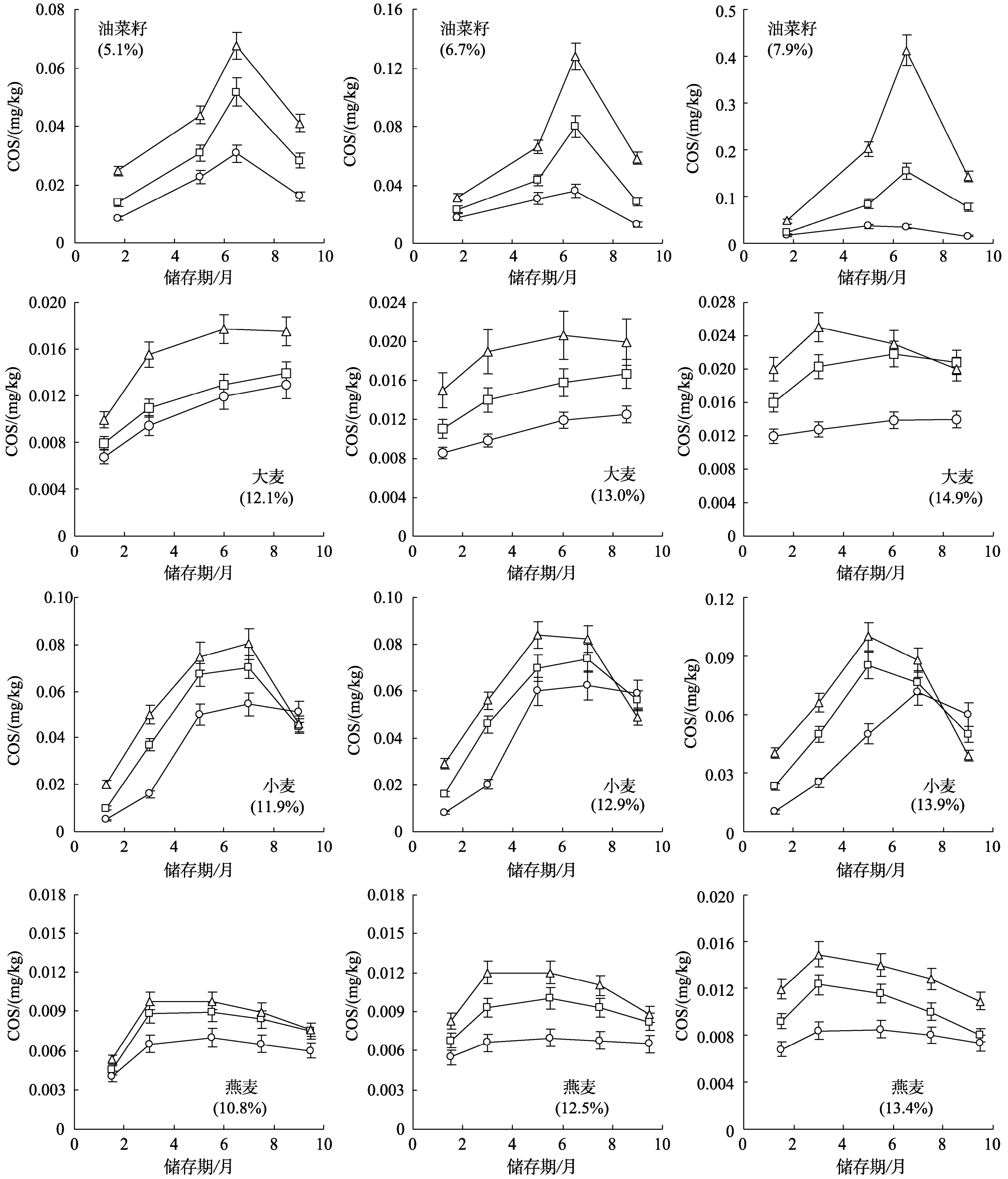
## 2　结果与分析

### 2.1 产品中硫化羰的本底水平

硫化羰自然存在于所有新收获的谷物和油料中，含量为 0.005~0.500 mg/kg，具体为小麦、大麦和燕麦的含量为 0.005~0.040 mg/kg；菜籽油的含量为 0.010~0.500 mg/kg。含量因品种、含水量、粮温和储存期而异（图 1）。含水量和粮温越高，硫化羰含量越高。对于小麦和油菜籽，储藏 5~7 个月时的浓度增加了 3~10 倍，然后开始下降，粮温和含水量越高越明显。对于燕麦，储藏前三个月硫化羰含量呈上升趋势，然后略有下降。然而，对于油菜籽，在 9 个月的储藏实验中，最高含水量（7.9%）或最高粮温为 35 ℃的样品硫化羰含量前六个月呈上升趋势，从第 6 个月开始下降，其他含水量和粮温梯度含量呈逐渐上升过程（图 1）。

### 2.2　产品中甲酸乙酯的本底水平

与硫化羰一样，甲酸乙酯也自然存在于新收获和储藏谷物，含量在 0.1~0.6 mg/kg，其中小麦和大麦为 0.1~0.4 mg/kg，燕麦为 0.2~ 0.4 mg/kg。含量因商品、储存温度、含水量和储存期而异（图 2）。对于油菜籽和燕麦，储藏 7 个月时，甲酸乙酯的浓度增加了 2~5 倍，然后开始下降，粮温和含水量越高越明显。15 ℃低温和低含水量时，10

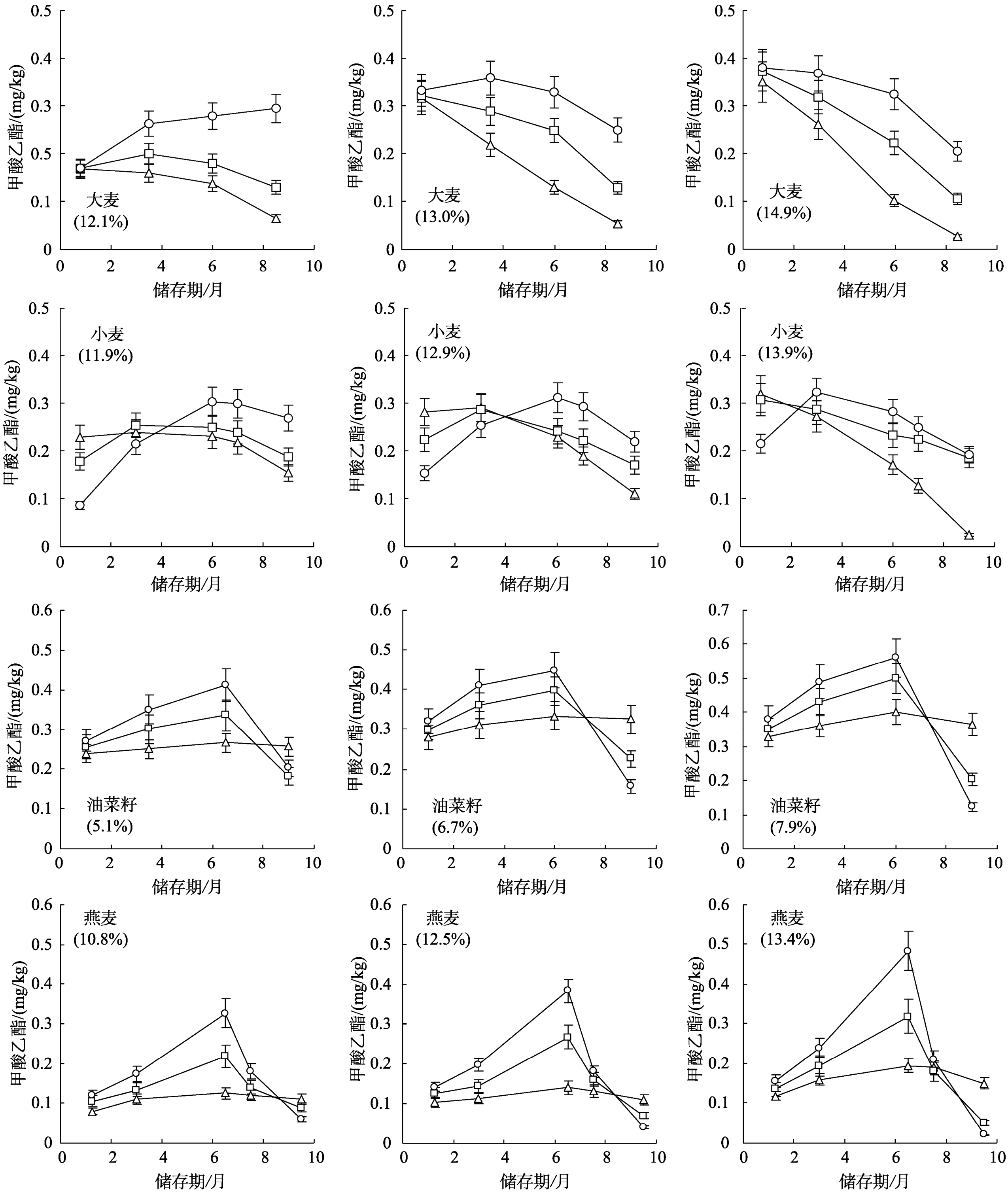


注：—O— 15 ℃；—□— 25 ℃；—Δ— 35 ℃。

图 1 不同温度、含水量和储藏时间下大麦、油菜籽、燕麦和小麦的硫化羰本底水平

Fig. 1 Natural occurrence of carbonyl sulfide in barley, canola, oats and wheat, at different temperature, moisture content and period of storage

个月的贮藏期间油菜籽和燕麦中甲酸乙酯含量均没有显著变化，分布为 5.1%和 10.8%（图 2）。对于小麦和大麦，当含水量分别为 11.9%~12.9%和 10.8%以及 25 ℃粮温时，甲酸乙酯的含量前 3 个月呈上升趋势，随后下降（图 2）。然而，当粮温较高时，如在 25 ℃和 35 ℃时，即使含水量较低，小麦和大麦中的甲酸乙酯含量从第一个月开始下降（图 2）。



注：—O— 15 ℃；—□— 25 ℃；—Δ— 35 ℃。

图 2 不同温度、含水量和储藏时间下大麦、油菜籽、燕麦和小麦的甲酸乙酯本底水平

Fig.2 Natural occurrence of ethyl formate in wheat, barley, oats and canola, at different temperature, moisture content and period of storage

# 3 讨论

## 3.1 产品中硫化羰的本底水平

硫化羰（COS）自然存在于种子中，但含量因植物种类和环境等因素而异。在新收获的谷物中检测到硫化羰，表明它是在植物发育过程中自然形成的。其形成有两个潜在的因素，一是植物生长过程中从环境中吸收，因为在植被和土壤的

排放物中发现了大量的硫化羰[29,34-36]，生物生产量越多的地区含量越高[28,37]。排放硫化羰含量随有机肥施用量的增加而增加，且与土壤总硫含量[38]和植被种类[36]呈正相关。二是酶促反应或生物水解，即微生物活动或生物合成产生的防御性产物[29,39-42]。细菌产生硫化羰已得到充分证实，谷物表面存在的特定细菌也会导致硫化羰含量升高[43]。进一步研究硫化羰的生物学作用，并将硫化羰的化学生物学与其他因素区分开来，是将来研究的一个方向。

硫化羰存在于所有储存谷物中，储藏期的前 3~9 个月，硫化羰含量呈上升趋势，这可能于谷物本身代谢和微生物作用有关。此外，硫化羰增长速度不是恒定的，受到特定条件的影响。其中，粮温和含水量等因素起着关键作用。通过研究了解这些因素的作用有非常重要的意义，因为它们与硫化羰含量直接相关，从而影响到这些产品的储存和货架期。这些结果与在制麦芽和酿造过程中存在天然硫化羰的研究发现一致[44]。本研究中检测到的硫化羰水平与之前实验室和现场实验研究结果一致，即小麦中含量为 0.005~0.100 mg/kg，大麦为 0.005~0.050 mg/kg，燕麦为 0.005~0.030 mg/kg，油菜籽为 0.02~0.60 mg/kg[13,19,30-31]。

### 3.2 产品中甲酸乙酯的本底水平

在新收获谷物中检测到甲酸乙酯，证明在植物生长和发育期间就自然存在，由商品和存在的微生物的代谢过程产生的。含水量对其本底水平有影响，且与粮温也相关。这些结果与 Desmarchelier 等[19]报道的大麦中甲酸乙酯本底水平一致，他们发现在高温储存条件下，甲酸乙酯含量从 1.0 mg/kg 降至 0.2 mg/kg。这些结果也与在微生物中天然存在甲酸乙酯的发现一致，如意大利青霉和指状青霉，它们随着含水量和温度的增加而增加[45]。在实际情况下，如果在高含水量和高温下的商品中未发现甲酸乙酯，说明其已经分解为乙醇和甲酸。Yuen 等[45]指出，当甲酸乙酯的天然浓度较高且暴露时间较长时，甲酸乙酯可能具有抗菌特性。利用甲酸乙酯可以抑制其在微生物中进一步产生，是未来研究的一个关键领域。这种现象可能解释了在储存期后观察到的甲酸乙酯水平下降，尤其是在粮温和/或含水量升高的情况下。明确这一作用，对未来研究甲酸乙酯具有重要意义。

## 4 结论

在收获和储存期间，硫化羰和甲酸乙酯都会自然存在于谷物中，说明硫化羰和甲酸乙酯都是在谷物田间生长和储存过程中产生的，可能是由谷物及相关微生物的代谢引起的。这似乎是一个标准反应，以致于谷物表现出不同含量的硫化羰和甲酸乙酯。谷物中硫化羰和甲酸乙酯本底水平的自然变化与任何传统毒性剂量或健康风险无关。从剂量和安全的角度来看，含有这个浓度硫化羰和甲酸乙酯的谷物被认为是正常的。因此，在设定这些物质的最大残留限量（MRL）时，了解这些本底水平至关重要，因为这可作为市场接受硫化羰和甲酸乙酯作为储存产品熏蒸剂的评价因素。即，通过检测自然存在的这些，可指导其在谷物储存中使用相关法规制定，提高公众认知。

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英文原文详见 P24-29。

KOLI P, MAHAWAR S, XIAO Y, et al. Evaluation of background levels of ethyl formate and carbonyl sulfide in grains and oilseeds (英文原文)[J]. Science and Technology of Cereals, Oils and Foods, 2024, 32(3): 16-29.

# Evaluation of Background Levels of Ethyl Formate and Carbonyl Sulfide in Grains and Oilseeds (英文原文)

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**Abstract:** Ethyl formate and carbonyl sulfide are potential stored product fumigants. They are present naturally in the atmosphere, plants and soil. Natural levels of ethyl formate and carbonyl sulfide in newly harvested grain and stored wheat, barley, oats and canola are varied with the commodity, temperature, moisture content and the period of storage. The values ranged from 0.5~2.0 mg/kg for ethyl formate and 0.02~1.0 mg/kg for carbonyl sulfide. Ethyl formate and carbonyl sulfide were naturally present in grains at harvest, increased during the first 4~5 months of storage, and then began to decline, particularly at grain temperatures greater than 20 ℃ and moisture contents of grain and canola greater than 9.5% and 5%. This information on natural levels is relevant to the establishment of Maximum Residue Limits (MRL) and to market acceptance of ethyl formate and carbonyl sulfide as fumigants for stored products.

**Key words:** ethyl formate; carbonyl sulfide; fumigant; background level of fumigant; grains; oilseeds

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## 1 INTRODUCTION

Ethyl formate (EtF) and carbonyl sulfide (COS) are both evaluated as fumigants for the control of pests and pathogens in stored agricultural products[1-8].

Ethyl formate has a long history of use as a fumigant for dried fruit[6,9-10] and other stored products[11-12]. The work safety related TLV is 100 ppm, which is the maximum concentration that a person is allowed to be exposed to daily for a working lifetime shows that EtF is much safer than all other fumigant, such as TLV for phosphine ($PH_3$) is 0.3 ppm and methyl bromide (MeBr) is 1 ppm. Ethyl formate has been re-evaluated by Desmarchelier *et al.*[13] as an alternative to MeBr for stored grain and recent studies found its use on in paddy rice[14], quality of Barley[15], chestnut[16]. Furthermore, its studies have been conducted on fruit qualities like in citrus[17]and blueberries[18]. Recently, ethyl formate has been successfully used as a fumigant for control of invertebrate pests in general cargo with packaged food, drunk and dried fruits without effect of food quality[6]. Ethyl formate is a naturally occurring volatile present in the environment air, milk, cheese,

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wheat, beer, and many fresh products[19]. It is rapidly hydrolyzed to ethanol and formic acid which are also biogenic compounds[20]. Ethyl formate is generally regarded as safe (GRAS) as a food addictive[21] and is used as a flavoring agent with no evidence that it is hazardous to the public[22]. Natural levels of EtF in a range of products, such as vegetable, stored grain and animal products, have been reviewed by Desmarchelier[19]. It is a naturally occurring insecticidal compound that offers potential as an alternative to synthetic insect fumigants, which can be harmful or detrimental to the environment[18]. For example, EtF as a naturally occurring volatile is present in the following products: 0.15 mg/kg in milk[23]; 1.3 mg/kg in cheese[24]; 0.3 mg/kg in wheat and 0.03~0.7 mg/kg in wheat germ[13]; 0.2~1.0 mg/kg in barley[19]; and 0.9~10 mg/kg in barley products such as beer [25].

Carbonyl sulfide (COS) is regarded as one of the potential alternative fumigants to replace MeBr[26-27]. In regarding as alternative source to MeBr and $PH_3$, COS was also shown prominent activity against phosphine resistant insect *Tribolium castaneum* without significant changes in the quality of rice grain[5]. Natural levels of EtF in a range of products, such as vegetable, stored grain and animal products, have been reviewed by Desmarchelier[19]. It is a naturally occurring insecticidal compound that offers potential as an alternative to synthetic insect fumigants, which can be harmful or detrimental to the environment[18]. Similarly, many natural sources of COS have been identified, including oceans, soils, volcanoes and marshes[28-29]. Therefore, COS is naturally present in the atmosphere, in water, soil, and plants, as well as many raw and processed foodstuffs, including cereals and oilseeds. The natural levels of COS in grains and oilseeds were found to be 0.02~0.07 mg/kg[13,30-31].

The Australian Pesticides and Veterinary Medicines Authority (APVMA) does not specify ethyl formate's MRL for the stored commodities, as it considers the residues should be identical to those commonly found in untreated food and therefore is of no toxicological significance[32], but no systematic research evidence to support this consideration. There is little information on the effect of temperature, moisture content, variety and the period of storage on natural levels of EtF and COS in stored grain. As part of a study to evaluate EtF and COS as grain fumigants, information about the natural occurrence of the fumigants in stored products is relevant to the establishment of Maximum Residue Limits (MRL) and to the acceptance of EtF and COS as fumigants of stored products. Therefore, it is important and urgently need to conduct systematic research with known historical grain and oilseed samples for understanding the background level of EtF in grain and oilseed and its dependent factors, such as period of storage, temperature and moisture content.

In this report, we have evaluated the effect of temperature, moisture content, commodity and the period of storage on the natural levels of COS and EtF in Australian wheat, barley, oats and canola. Selection of the grains was based on the fact that wheat is the major cereal grain in Australia, and barley because it is the second major cereal grain, where quality is especially important to the brewing industry. Oats were selected because their composition is different from other cereals, with a lipid content of 5.5%~6.5%, which is 2~3 times higher than that of wheat, barley or rye, and a fibre content of 11%~12%, 2~4 times higher than in most cereals[33]. Canola was selected due to it being the principal oilseed in Australia.

## 2 MATERIALS AND METHODS

### 2.1 Preparation of the commodities

Four Australian representative grains (wheat, oats, barley and canola) were used. They were collected by hand, prior to harvesting to exclude any possible contamination. All commodities (1.5 kg) were placed into sealed jars (2 L), and a varying amount of distilled water was added to the jars to adjust the moisture content. They were allowed to equilibrate for 2 weeks at three different temperatures, (15±1) ℃, (25±2) ℃ and (35±2) ℃, and the moisture contents (wet basis) were measured following the procedures of the International Standard (ISO 712—2015). The moisture contents were: 11.9%, 12.9% and 13.9% for wheat; 12.1%, 13.0% and 14.9% for barley; 10.8%, 12.5% and 13.4% for oats; and 5.1%, 6.7% and 7.9% for canola. For each moisture content grain condition, the conditioned grain was divided into 3 (2L) jars and sealed for the storage period at (15±1) ℃, (25± 2) ℃ and (35±2) ℃. Samples were tested for EtF and COS levels at intervals up to 9 months of storage.

### 2.2 Reagents and apparatus

A cylinder of compressed COS gas was

purchased from Matheson Gas Company, USA, via BOC, Australia. All the reagents used were analytical grade. Acetone was supplied by Biolab Scientific, Clayton, Victoria, Australia. Ethyl formate was supplied by Tech Ajax, Sydney, Australia. All other chemicals were obtained from BDH AnalaR, England. Extractions were carried out in 250 mL Erlenmeyer flasks with a ground-glass joint (Crown Scientific, N.S.W, Australia, Cat. No. FE250/3) fitted with a Quik Fit stopper containing a half hole septum (Alltech Associates, P/N 6526).

### 2.3 Determination of natural levels of carbonyl sulfide and ethyl formate

Carbonyl sulfide was determined on a Shimadzu GC6AM GC (Shimadzu Seisakusho, Kyoto, Japan), equipped with a flame photometric detector (FPD). Separation was achieved on a 1 m × 3 mm (i.d.) glass column packed with HayeSep Q (Alltech Associates, Cat. No. 2801) at 140 ℃ and carrier flow ($N_2$) of 40 mL/min at 0.8 psi. Concentrations of ETF were determined using a Varian Star 3600 CX series (Varian Associates, lnc., USA), equipped with a flame ionisation detector (FID), after separation on a 30 m × 0.53 mm (i.d.) megabore capillary column DB FFAP (J & W 125-3212) at an oven temperature of 70 ℃.

The natural levels of COS and EtF were determined by analysis of the headspace over the commodity plus the extraction solvent, following the procedure described by[13] Desmarchelier *et al.*, and Le and Ren (personal communication). For analysis of COS, the commodities (50 g) were extracted in sealed flasks (250 mL) with 50 mL of acidified aqueous acetone ($H_3PO_4$+water+acetone, 1 : 2.2 : 12.5, v/v/v) solution for 10 hours. For analysis of EtF, the commodities (50 g) were extracted in sealed flasks (250 mL) with 70 mL of ammonium nitrate (70%, w/v) solution for 24 hours. An aliquot of the headspace (50 μL) was injected directly into the GC, and the levels of COS or EtF were calculated on the basis of peak areas. Periodically, the peak areas were calibrated using a spiked standard. The data recorded in the figures are the mean of duplicate samples.

### 2.4 Preparation of the gas standards, and the fortified samples

A dilute gas was prepared by injecting a measured volume of the concentrated gaseous COS or the liquid ETF into a flask (1 L) containing five glass beads (2~3 mm o.d.). After mixing, the diluted gas was used to prepare both the fortified samples and the gas standards. The spiked samples, carried out in duplicate, were prepared by injecting a measured volume of the diluted gas into a sealed flask containing the commodity plus the extraction solution. For the Gas Chromatograph analysis of COS, complete elution of solvent or solvent vapour was required before further injection, so a minimum interval of 10 min was kept between injections.

## 3 RESULTS

### 3.1 Natural levels of carbonyl sulfide in the commodities

Carbonyl sulfide was found to be naturally present in Australian wheat, barley, oats and canola during the 9~10 months of storage, but levels varied with commodity, moisture content, temperature and the period of storage (Figure 1). The levels of COS were greater at higher moisture contents and high grain temperatures. The values ranged from 0.005 mg/kg to 0.500 mg/kg of COS in all the tested cereal grains and canola (Figure 1). Carbonyl sulfide occurred naturally in all the newly harvested grains, eg. 0.005~0.040 mg/kg in wheat, barley and oats; 0.010~0.500 mg/kg in canola. In wheat and canola concentrations increased 3~10 times during the 5~7 months of storage, and then began to decline, particularly, when the grain temperature and moisture content were rising. Carbonyl sulfide in oats increased during the first 3 months of storage, and then declined slightly. However, in canola COS levels increased during 9 months of experimental storage, except in the samples with the highest moisture content (7.9%) or the highest grain temperature of 35 ℃ In both cases COS declined after 6 months of storage (Figure 1).

### 3.2 Natural levels of ethyl formate in the commodities

Ethyl formate was found to be naturally present in Australian wheat, barley, oats and canola during the 10 months of storage. Levels varied with the commodity, temperature of storage, moisture content and the period of storage (Figure 2). The values ranged from 0.1~0.6 mg/kg of EtF in all the tested grain samples. As with COS, EtF also occurred naturally in the newly harvested grain, eg. 0.1~0.4 mg/kg in wheat, barley and oats; and 0.2~

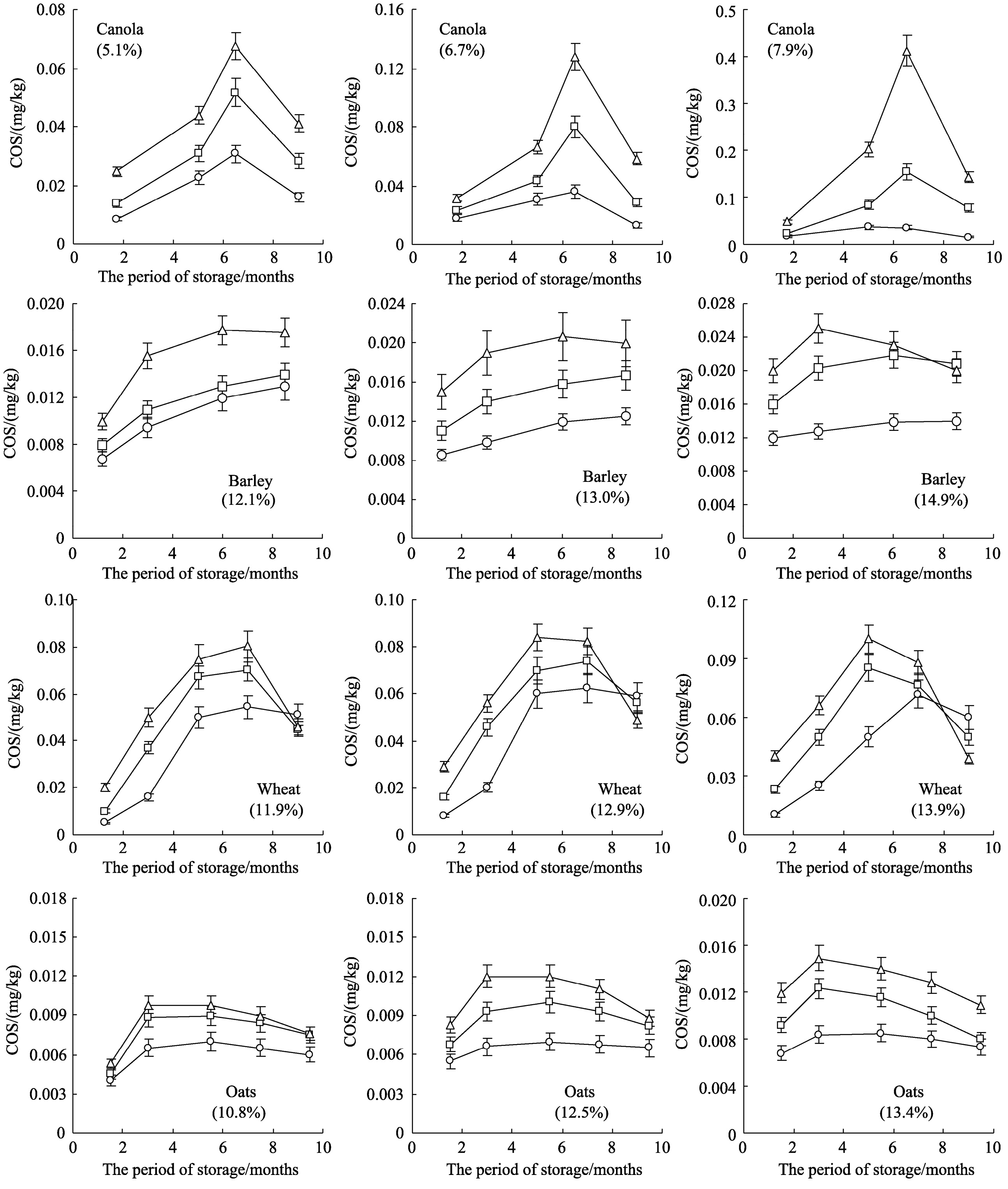

**Fig.1 Natural occurrence of carbonyl sulfide in barley, canola, oats and wheat, at different temperature, moisture content and period of storage (—O—, at 15 ℃; —□—, at 25 ℃; —Δ—, at 35 ℃)**

0.4 mg/kg in canola. Ethyl formate in canola and oats increased 2~5 times during the first 7 months of storage, and then began to decline, particularly, when grain temperature and moisture content were rising. At 15 ℃ grain temperature and low moisture content, such as 5.1% m.c. in canola or 10.8% m.c. in oats, the background levels of EtF in canola and oats had no significant change during the 10 months storage (Figure 2). However, at the same temperature in wheat and barley, the EtF background levels did increase during the 3~9 months of storage. At a moisture content of 11.9%~12.9% for wheat and

10.8% for barley, and at 25 ℃ grain temperature, the EtF background levels also increased during the first 3 months of storage, but then declined (Figure 2). However, in both wheat and barley, when the grain temperature was higher, such as at 25 ℃ and 35 ℃, the EtF background levels decreased from the first month of storage, even with a low moisture content (Figure 2).

# 4 DISCUSSION

## 4.1 Natural levels of carbonyl sulfide in the commodities

Carbonyl sulfide (COS) can be naturally present in seeds for various reasons, although specific reasons can vary based on the type of plant, environmental factors, and more. COS has been detected in freshly harvested grains, indicating that it naturally forms during plant development. There are potentially two reasons for its formation. The first is through absorption as the plant grows. Notably, significant quantities of COS have been identified in emissions from both vegetation and soil[29,34-36] and it is much greater from areas of high biological productivity[28,37]. Emission of COS increased with the application of organic manure, and it was positively correlated with the total sulfur content in the soil[38] and the types of vegetation[36]. The second is through enzymatic synthesis or biological hydrolysis through microbial activities or biosynthesis during the defense[29,39-42]. Bacterial production of COS is well-established, and specific bacteria present on grain surfaces also contribute to COS levels[43]. Moving forward, it's essential to further investigate the biological roles of COS and to differentiate the chemical biology of COS from other factors.

Carbonyl sulfide consistently appears in all stored grains, with its presence increasing within the initial 3~9 months of storage. This escalation in COS levels is likely a consequence of metabolic processes and the actions of microorganisms within the grain. Furthermore, the pace at which COS increases is not constant and appears to be influenced by specific conditions. Notably, factors such as the temperature of the grain and its moisture content play pivotal roles in determining this rate of increase. It's crucial to understand the dynamics of these factors, as they seem to have a direct correlation with COS concentrations, which could have implications for the storage and longevity of these commodities. These results are consistent with those reported for naturally occurring COS present during the malting and the brewing processes[44]. The levels of COS observed in all the examined grains align with findings from prior laboratory and field trial research. They were 0.005~0.100 mg/kg in wheat, 0.005~0.050 mg/kg in barley, 0.005~0.030 mg/kg in oats, and 0.02~0.60 mg/kg in canola[13,19,30-31] .

## 4.2 Natural levels of ethyl formate in the commodities

The presence of EtF in freshly harvested grains demonstrated that it occurs naturally during plant growth and development. The effect of moisture content on its natural levels is associated with the grain’s temperature. This indicates that naturally occurring EtF results from metabolic processes in both the commodities and the microorganisms present. The results are consistent with the natural levels of EtF in barley reported by Desmarchelier *et al*[19]., who found that levels reduced from 1.0 mg/kg to 0.2 mg/kg under high temperature storage conditions. The results are also consistent with naturally occurring EtF found in micro-organisms, such as *Penicillium italicum* and *P. digitatum*, which are reported to have increased with increasing moisture content and temperature[45]. In fact, if no EtF found in the commodities at high moisture content and high temperature, this is due to its break down to ethanol and formic acid. Yuen *et al*[45]. have pointed out that EtF may have antimicrobial properties when concentrations of natural levels of EtF are high and exposure time is long. The fact that introducing EtF can inhibit its further production in microorganisms suggests a crucial area for future investigation. This phenomenon might explain the observed reduction in EtF levels following a storage period, especially in conditions where grain temperature and/or moisture content are elevated. The understanding of this mechanism underscores the importance of EtF in future research endeavours.

# 5 CONCLUSIONS

During harvest and storage periods, COS and EtF are naturally present in stored grains. Both COS and EtF are produced during the grain's growth in the field as well as during storage, likely due to the metabolism of the grain and its associated microorganisms. This seems to be a standard procedure, resulting in grain that exhibits varying levels of COS and EtF. The natural variations

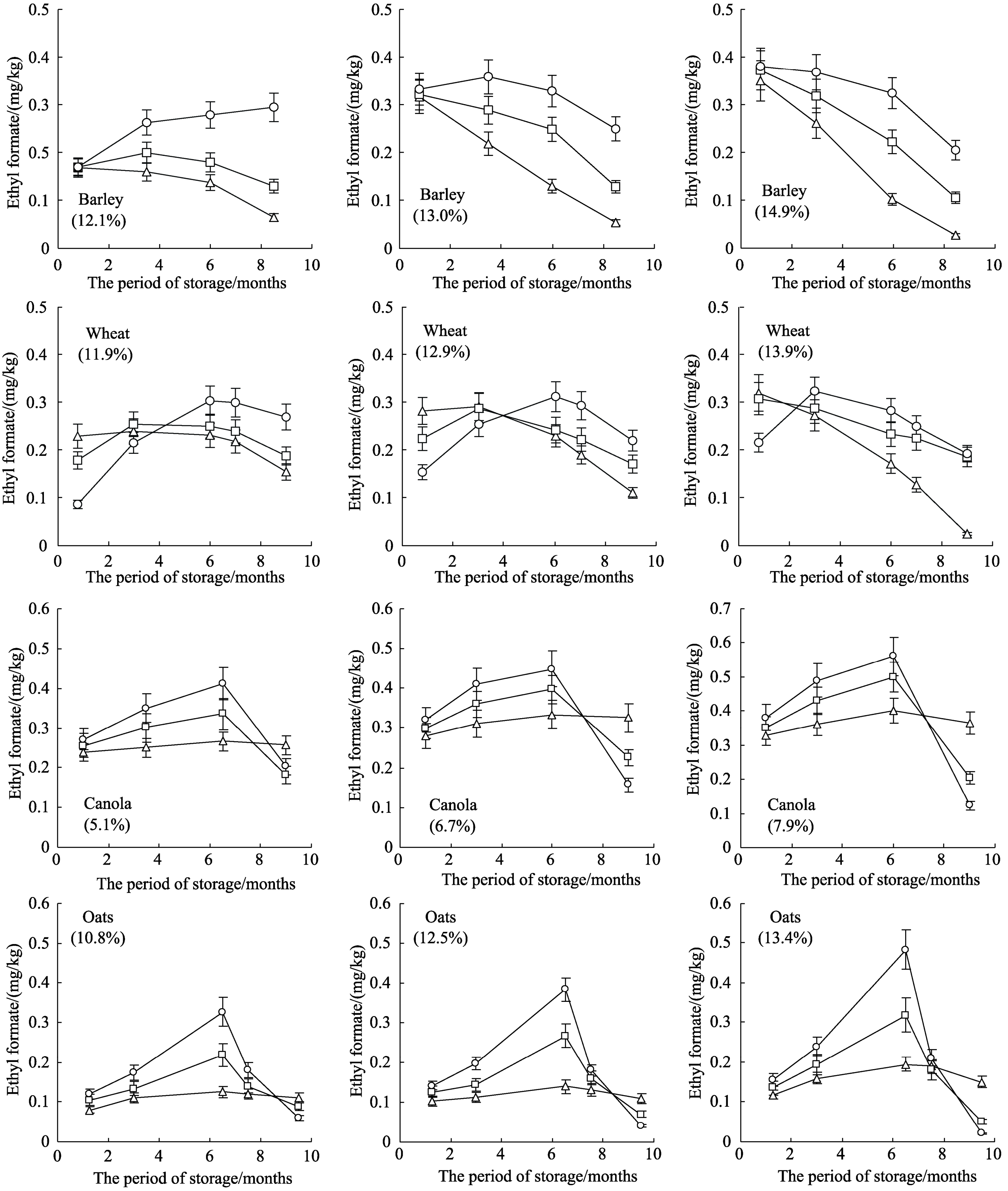

**Fig.2. Natural occurrence of ethyl formate in wheat, barley, oats and canola, at different temperature, moisture content and period of storage (—O—, at 15°C; —□—, at 25°C; —Δ—, at 35°C)**

in the levels of COS and EtF present in grains are not linked to any traditional quality attributes or known health risks. From a quality and safety perspective, grains with these natural levels of COS and EtF are considered normal. Understanding these natural levels is crucial when setting Maximum Residue Limits (MRL) for these substances. Moreover, this knowledge is pertinent to the market's acceptance of COS and EtF as potential fumigants for stored products. In other words, by recognizing what's naturally present, it can guide regulations and public perceptions related to using these compounds in grain storage.

## REFERENCES

See in its Chinese version P21-23.