

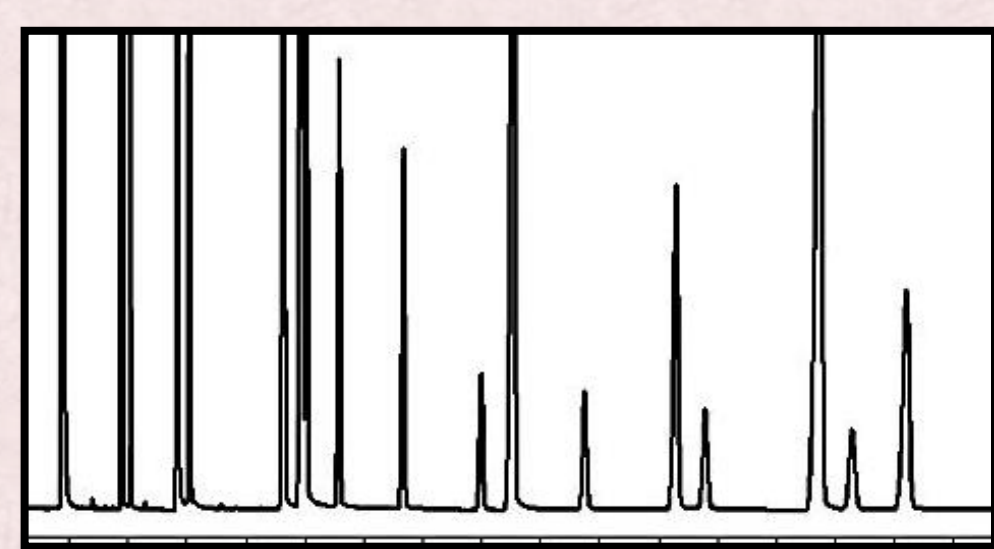
Idea of rapid preparation of fatty acid methyl ester using *in situ* derivatization from fresh horse mussel

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Introduction

Fatty acid profile



- proof of origin & authenticity
- analyze essential/special function FA

GC analysis after derivatization as fatty acid methyl ester (FAME)

Problem of single catalyst

Consecutive use of catalysts

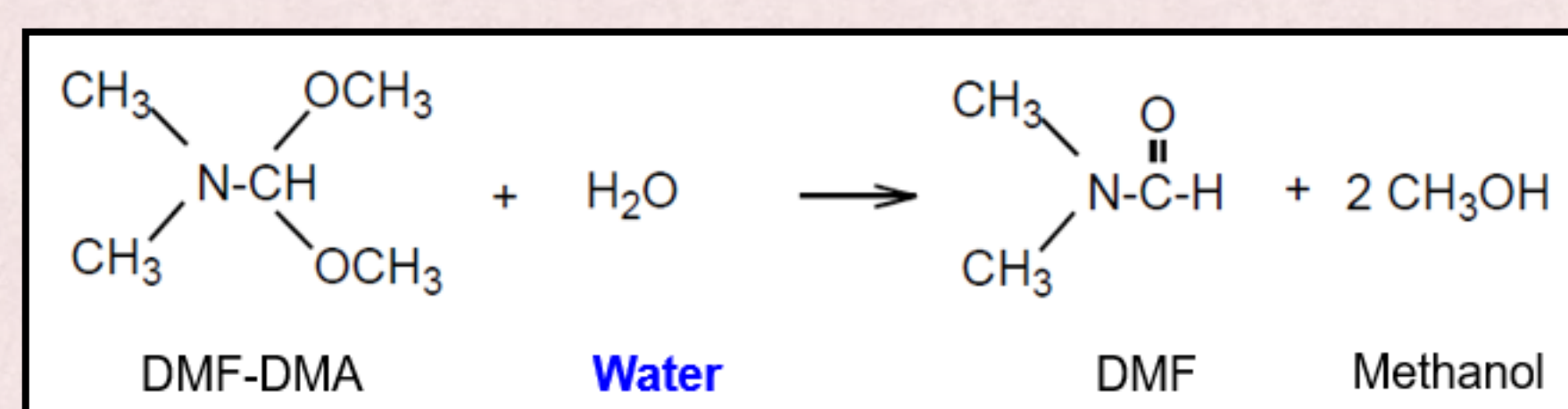
- ✓ Moisture in sample
- ✓ FFA in sample
- ✓ Reduce time
- ✓ Without pretreatment

Preparation technique [1]

1. Traditional method

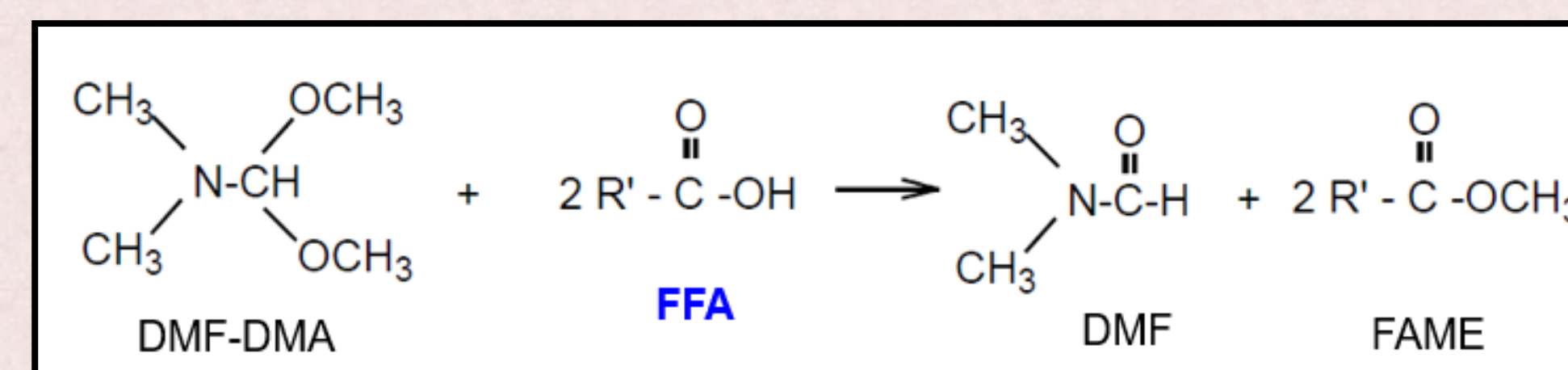
- 1) Oil extraction
- 2) Derivatization
 - Saponification and methylation
 - Transesterification

2. *In situ* method



Catalyst performance comparison

Features	Acid catalysis	Base catalysis
Temperature	100 °C	25- 50 °C
Reaction time	1-2 h	1-10 min
Esterifying power	High	No
Transesterifying power	Low	High
Sensitive to water	Low	High
Artifact/isomerization	Yes	No



DMF-DMA reaction scheme [2]

Results

Table 1 Lipid composition and moisture content of fresh horse mussel

lipid composition (% of total extracted oil)	
triglyceride	80.1 ± 2.1
free fatty acid	14.3 ± 0.1
other ¹	5.6 ± 2.3
content (% of fresh sample)	
moisture	75.11 ± 0.24

¹ di-glyceride and unidentified peaks

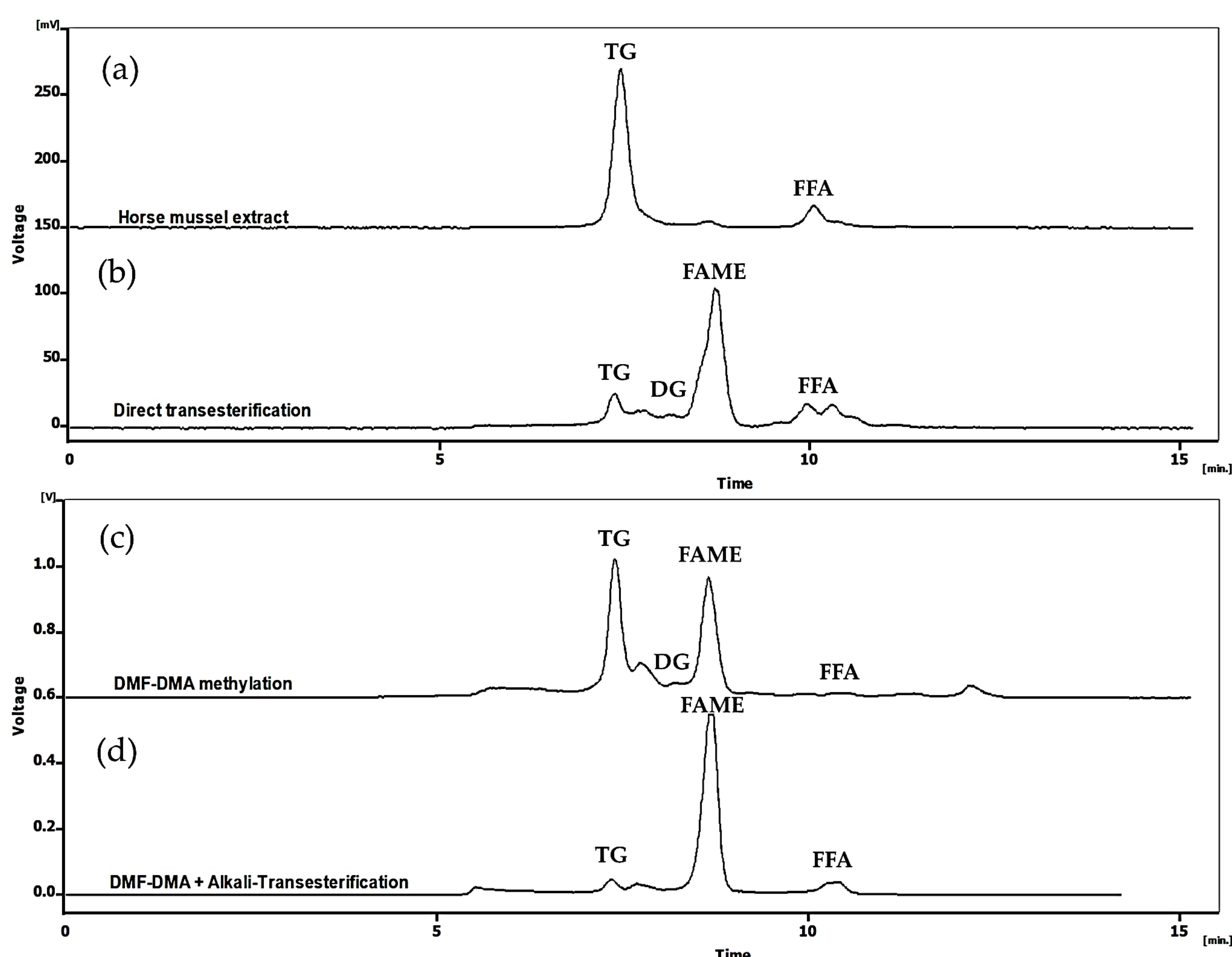


Fig. 2 HPLC chromatogram of (a) extracted horse mussel oil, products from (b) direct alkali-*in situ* transesterification method, (c) DMF-DMA *in situ* methylation method and (d) 2 steps method

Methods

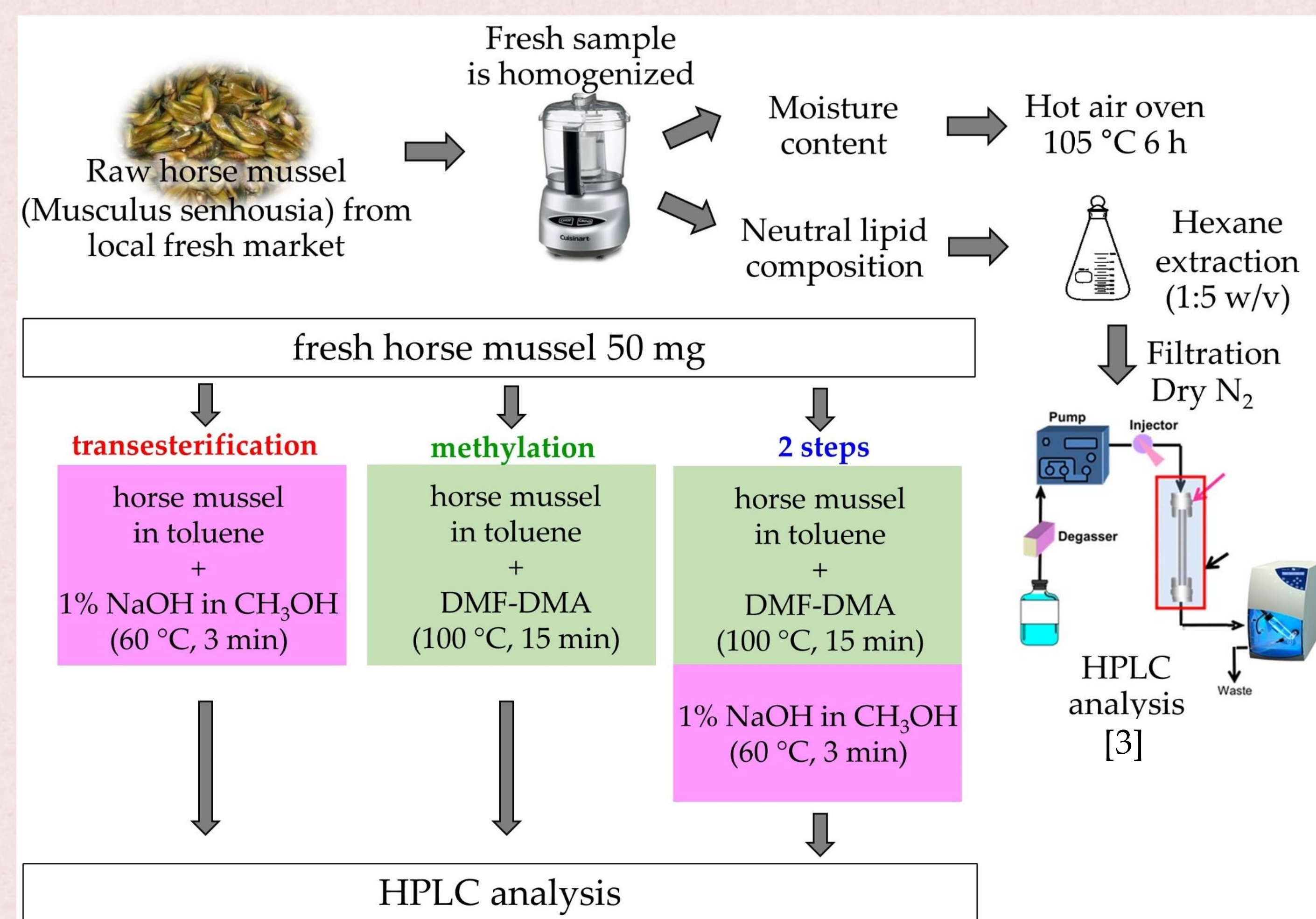


Table 2 Products (% of total oil) by direct alkali-*in situ* transesterification method and DMF-DMA *in situ* methylation method in comparison with 2 steps method

compound	direct alkali- <i>in situ</i> transesterification	DMF-DMA <i>in situ</i> methylation	2 steps method
FAME	58.7 ± 1.8	40.2 ± 1.5	79.9 ± 0.0
triglyceride	12.4 ± 4.9	44.8 ± 6.7	7.8 ± 4.0
free fatty acid	21.1 ± 0.4	1.9 ± 0.4	8.5 ± 0.7
other ¹	7.9 ± 6.4	13.3 ± 5.6	3.8 ± 4.7

¹ di-glyceride and unidentified peaks

Conclusion

- The two steps *in situ* derivatization; methylated with DMF-DMA, followed by alkali-transesterified showed the FAME yield of 79.9% within 18 min of reaction.
- The proposed method could be an alternate derivatization technique for FAME preparation from fresh sample containing high moisture and FFA.

References

- [1] Eder, K. (1995). J. Chromatogr. B Biomed. Appl., 671(1-2), 113-131.
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Acknowledgement

The authors acknowledge King Mongkut's University of Technology Thonburi for funding the KMUTT Research Center of Excellence Project to Lipid Technology Research Group.