CROMATOGRAPHIC CONDITIONS TO OPTIMIZE THE SEPARATION OF FATTY ACID METHYL ESTERS FROM BOVINE MEAT UTILIZING THE HIGH POLAR SLB-IL111 COLUMN

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Abstract –The objective of this study was to develop a gas chromatographic method (CM) utilizing a high polar capillary column able to improve the separation of relevant fatty acids as methyl esters (FAMEs) from bovine meat. The new method, named "Camaquã-v7" (CV7), was compared to three other methods: the method suggested by the manufacturer (SIG), an isothermal method of 180 ºC-60 min (IS8) and an isothermal method of 168 ºC-90 min. The four CM were compared based on the ability of each method to separate the FAMEs in the GLC-674 standard and those obtained from one sample of bovine beef. Four replicates were utilized for each sample within each CM. The CV7 method provided separation of all 52 FAMEs present in the standard. The improvement in FAMEs separation from bovine beef lipid fraction was even larger: 75^{c}; 83.5^{b}; 81.5^{b} and 98.5^{a} peaks were separated, respectively in SIG, IS8, IS68 and CV7. The CV7 method provided a greater separation of FAMEs, whereas CV7 run time was shorter than IS8 and IS68.

I. INTRODUCTION

There is an increasing concern from research and industry in the fatty acid (FA) composition of animal products. Complete separation of FA in ruminant fat is a challenge due to the large diversity of fatty acids, particularly mono and dienoic isomers, as well as branched chain FA (1). The development of new chromatographic columns over the years provided gradual improvement in separation of FAMEs, however, the separation of mono and dienoic isomers are still a challenge. Recently, a new stationary phase column was released in the market with the objective of improving the separation of such FA isomers. Delmonte and colleagues (2011) published a detailed evaluation of the SLB-IL111, a polar cyanopropyl siloxane coated stationary phase column. These authors utilized the isothermal approach to identify the best temperature to improve the separation of target FA groups. Separation of C18:1 isomers were optimized at 168 ºC and 180 ºC, whereas conjugated dienes were best separated at 140 ºC. The objective of this study was to develop a chromatographic method utilizing a high polar capillary column able to improve the separation of quantitatively relevant FA as methyl esters from bovine meat in one single run, compared to methods already available.

II. MATERIALS AND METHODS

The CM CV7 was developed based on the maximum possible separation of FAMEs from a commercial standard that contained 52 FAMEs (GLC-674, Nu Chek Prep. Inc., Elysian, USA). Comparison of different CM was conducted in a gas chromatograph with mass spectrometer detector (GCMS-QP2010 Ultra, Shimadzu Co., Nakagyo-ku, Japan). The GCMS utilized helium as the carrier gas, and was equipped with a high polar capillary column (100m x 0.25mm x 0.2µm, SLB-IL111, Supelco, Bellefonte, PA, USA). The injection volume of 1µl of sample or standard, injector temperature of 250 ºC, and a split ratio of 1:25 were maintained for all CM. Methods were compared based on separation of FAMEs from a commercial standard (GLC-674, 7.8 mg/ml) and FAMEs obtained from the lipid fraction of a sample of bovine meat. The lipid fraction was extracted from bovine meat utilizing a modified version of the Bligh & Dyer (1959) method. Briefly, 0.6g of wet meat was washed with 9 ml of a solution of chloroform and methanol (2:1), centrifuged and the supernatant was separated. The sample was washed a second time with 3.6 ml of solution and 0.96 ml of distilled water, centrifuged, and the supernatant was removed. Chloroform (4.2 ml) and 4.2 ml of 0,88% KCl solution were added to the supernatant tube and mixed. After phase separation, aqueous phase was removed and solvents evaporated. The residue was methylated with a 3M KOH solution at 21 ºC for 15 min. The FAMEs were extracted with isooctane and placed in a vial for GC injection.

The SIG method utilized a temperature program of 8 ºC/min. from 140 ºC to 180 ºC, followed by 5 ºC/min to 240ºC and 10 min. at 240 ºC, and linear velocity of 40 cm/sec. The methods IS8 e IS68 the temperature was maintained at 180 and 168 ºC for 60 e 90 min., and flow of 1.6 ml/min, respectively. The CV7 method utilized a temperature program of 3 ºC/min from 140 ºC to 180 ºC, isothermal condition at 180 ºC for de 11min, followed by 7 ºC/min to 240 ºC and 1min. at 240 ºC. The anova test was utilized for statistical analyses.

III. RESULTS AND DISCUSSION

The CV7 method was the only method evaluated that was capable of separating all 52 FAMEs present in the GLC-674 standard, whereas the IS68 method provided also a good separation, separating 51 FAMEs (Table 1). In addition, the CV7 method provided the best separation with a running time that was less than a half of the running time for the IS68 method ($P<0.01$).

Table 1. Means of four injections of GLC-674 (52 FAMEs) utilizing the different gas chromatographic methods.

	CV7	SIG	IS68	IS8
Peaks (n)	52	49	51	48
RT[1] C22:6 (min)	32.82[c]	16.03[d]	68.66[a]	40.97[b]
TIC[2] (x10[6])	3,200	1,921	3,551	3,316

[1]RT, retention time of last FAMEs peak; [2]TIC, total ion count.

The lipid extract from bovine meat contained a much larger number of FAMEs. The total number of FAMEs identified in each method was 75[c]; 83,5[b]; 81,5[b] e 98.5[a] respectively for SIG, IS8, IS68 and CV7. Because of lower initial temperature as well as a lower rate of temperature increasing, the CV7 improved the separation of FAMEs at the beginning of the chromatogram and therefore less co-elution was observed for short and medium chain FA. Less co-elution resulted in a larger number of total FAMEs separated, such as C16:1 isomers. The separation of FAMES in the C18:1 region utilizing the CV7 method (Figure 1) was less optimized than observed with IS68 and IS80 (Figure 2). The IS8 showed the best separation for the C18:1 region as previously demonstrated by Delmonte et al. (2011). The co-elution of C18:1, t11 and C18:1, c6 with the CV7 method was large enough to prevent quantification when the GLC-674 was evaluated. However, because of lower concentration of C18:1, c6 and higher concentration of C18:1, t11 in meat, the quantification of C18:1, t11 was only slightly compromised with CV7 method. The chromatographic method developed (CV7) was suitable for the type of meat utilized providing the separation of 98 fatty acids as FAMEs, which was greater than the number of FAMEs separated with previously published methods. The use of isothermals is an interesting and useful way of optimizing separation for specific groups of FA. The best CM will depend on the purpose of the study, type of sample and the resources available.

Figure 1. Separation of C18:1 isomers available in the GLC-674 utilizing the CV7 method.

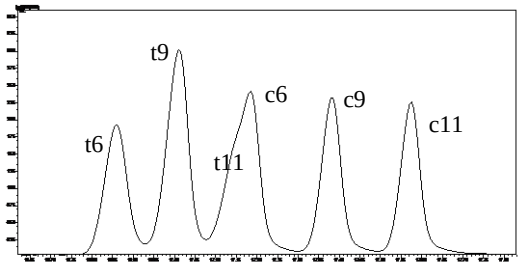
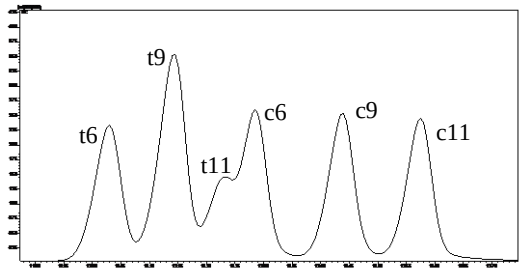


Figure 2. Separation of C18:1 isomers available in the GLC-674 utilizing the IS8 method.



IV. CONCLUSION

The CM presented in this publication, CV7, was successful in improving the overall separation of FAMEs in bovine meat lipid extract at a shorter running time, providing and interesting option for routine and research laboratories. This method was able to separate 98 FAMEs with a running time of 33.9 min.

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