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Research Article
A Rapid Method for the Determination of the Total Conjugated Linoleic Acid (CLA) Encapsulated in β-casein Nanoparticles

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Abstract

Background: β-casein was separated from different kinds of milk (Buffalo, cow, goat and camel) . Conjugated Linoleic Acid (CLA) was loaded in β-casein micelles. The micellar solution was frozen then lyophilized and stored in refrigerator. **Methodology:** A rapid method has been developed for the determination of encapsulated (CLA) in nano β-casein particles. A rapid method has been developed for the determination of encapsulated conjugated linoleic acid in nano β-casein particles. The method is based on the direct measurements of the absorbance of a solution of CLA loaded nanoparticles at 233 nm. **Results:** The determined CLA was highly correlated with initial concentration of CLA used in encapsulation and with results obtained by ethanol extraction. **Conclusion:** The method offers the advantage of eliminating the need for solvent extraction of entrapped CLA before spectrophotometric measurement.

Key words: Conjugated linoleic acid, nanoencapsulation, spectrophotometric measurements, β-casein

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Conjugated Linoleic Acid (CLA) is a group of linoleic isomers that proved to have several potential health effects[1]. Although there is recommended daily dose CLA, it is accepted normal diet has too low CLA content to get health benefits of CLA[2]. Enrichment of foods with CLA has been suggested to provide consumers with the required needs from CLA. Nowadays, CLA is industrially produced by alkali isomerization of linoleic acid. The product is mainly a mixture (1:1) of C18:2, 9c, 11t and C18:2, 10t, 12c. The CLA as other long chain fatty acids is almost insoluble in aqueous medium. Therefore, fortification of foods and beverages with CLA represent a challenge to food industry. Nanoencapsulation has been suggested as an efficient way to incorporate CLA in foods and to increase its bioavailability and stability.

Milk proteins have been used as nanodelivery systems for several hydrophobic bioactive food components such as omega-3 fatty acids[3], fish oil[4] but no cited literature o their use for encapsulation of CLA.

During the course for nanoencapsulation of CLA in β-case in there was a need for rapid method for the determination of CLA during encapsulation and storage of the prepared nanocapsules. Analysis of individual CLA isomers is one of the most complex and lime-consuming methods of fatty acid analysis[5] being unsuitable for analysis of large number of samples. Therefore, a rapid method for the determination of the total CLA is considered to be more appropriate to follow the behaviour of CLA during and after encapsulation in β-casein nanocapsules.

Spectrophotometric measurement of dienes at 233 nm has been used as rapid method for the determination of total CLA[6,7]. The objective of the present study was to develop a rapid method for the determination of CLA encapsulated in β-casein.

MATERIALS AND METHODS

Preparation of β-casein: β-casein was prepared from cow, buffalo, goat and camel skimmed milks by the method of Huppertz *et al.*[8] as shown in Fig. 1.

Preparation of CLA: Conjugated Linoleic Acid (CLA) was prepared from linoleic acid (Sigma, St., Louis, Mo) by alkali isomerization. The product had >90% CLA[9].

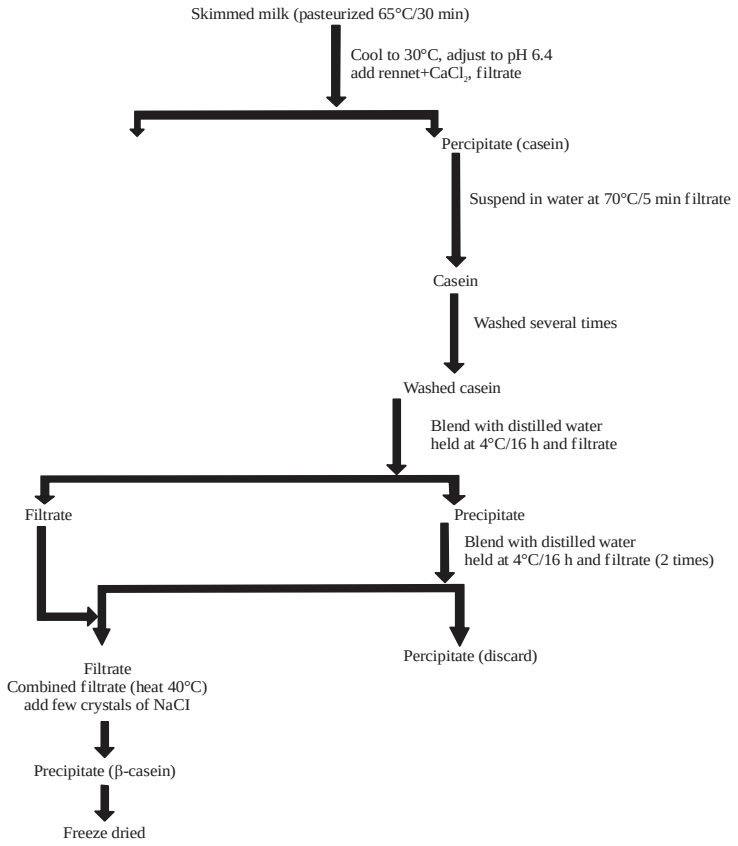

Fig. 1: β-casein isolation method

Preparation of CLA loaded β-casein nanoparticles: The CLA (25-100 mg/100 mg β-casein) was encapsulated in reassembled nano β-casein micelles as described by Zimet *et al.*[3] and freeze dried.

UV-absorption curves of CLA loaded β-casein nanoparticles: Ten milligrams of freeze dried CLA-loaded β-casein was dissolved in 5 mL of deionized water and the absorption spectra of the prepared solutions were measured at 200-400 nm using cary UV/Vis spectrophotometer (Agilent Technology, USA).

Determination of CLA: The CLA was determined by two methods, the 1st method based on the content of 10 mg freeze dried of CLA-loaded β-casein were extracted with 5 mL ethanol and the absorbance of the extract was measured at 233 nm and CLA concentration was determined from a standard curve of CLA prepared by plotting the absorption at 233 of standard pure CLA solutions (0.1-1 mg CLA mL^{-1}) in ethanol. The 2nd method is based on the determination of the absorbance of aqueous solution (10 mg/5 mL deionized water) of CLA-loaded β-casein at 233 nm and the concentration of CLA was determined from the standard curve.

Statistical analysis: Correlation analysis was done[10] between the used initial amount of CLA used in the loaded CLA-β-casein micelles and the determined CLA by the two methods used and between the results of the two methods.

RESULTS AND DISCUSSION

Figure 2 shows the absorption spectra of cow β-casein and CLA-loaded β-casein with 25, 50, 75 and 100 mg CLA/100 mg β-casein at 200-400 nm. A peak of maximum absorption was apparent at 233 in CLA-loaded β-casein which was not observed in β-casein solution. The intensity of the observed peak was increased with the increase in the entrapped CLA. Dienes including CLA are known to exhibit a strong UV absorption at 233 nm[11]. It is obvious that the β-casein matrix did not interfere with the UV spectra of the entrapped CLA. The aromatic side chains in the proteins only exhibit absorption at 280 nm and therefore, would not interfere with CLA maximum absorption. Absorption spectra of CLA loaded in buffalo, goat and camel β-caseins (results are not shown) were similar to that of cow β-casein.

The CLA contents entrapped in β-casein nanoparticles were determined from the measured absorbance at 233 nm in comparison to the prepared CLA standard curve. A total of 48 samples were analysed (three replicates from each level of CLA added to the four types of β-casein). The results are given in Table 1. In order to verify the relation between the initial concentration of CLA applied and the actual quantity entrapped correlation coefficient between the two variables were determined (Table 2). High positive correlations were found between the initial CLA concentration used in loaded β-casein and the determined CLA content by the two methods. Also, highly positive correlation was found between

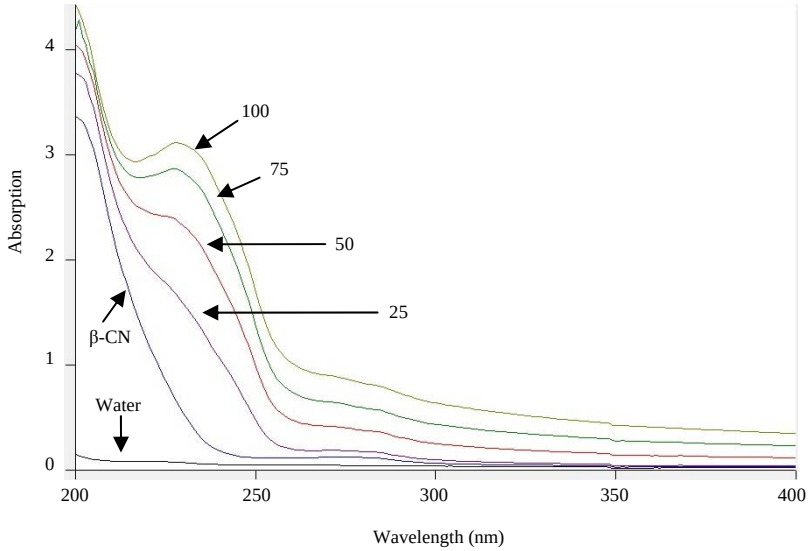

Fig. 2: UV spectra (in ascending order) of cow β-casein and CLA-loaded β-casein with 25, 50, 75 and 100 mg CLA/100 mg β-casein at 200-400 nm

Table 1: Initial CLA concentration used in loading β-casein and determined CLA content of loaded β-casein nanoparticles was determined by ethanol extraction method and direct UV method

Type of β-casein nanoparticles	Sample No.	Initial concentration (mg)	CLA content by ethanol extraction (mg)	CLA content by direct UV measurement (mg)
Cow	1	20	20	14
	2	44	39	31
	3	67	59	50
	4	87	82	66
Buffalo	1	21	15	18
	2	41	34	26
	3	65	51	48
	4	85	75	52
Camel	1	21	21	20
	2	44	44	28
	3	61	63	46
	4	82	68	54
Goat	1	22	18	21
	2	45	44	39
	3	64	53	48
	4	87	79	70

Table 2: Correlations between initial CLA and CLA determined by extraction and by the direct method of determination

Variables	r	r^2	Slope	Intercept	Standard error
Initial × extraction	0.98	0.96	0.89	0.27	4.36
Initial × direct	0.97	0.94	0.68	3.09	4.54
Extraction × direct	0.96	0.93	0.75	3.50	4.90

entrapped correlation coefficient between the two variables were determined (Table 2). High positive correlations were found between the initial CLA concentration used in loaded β-casein and the determined CLA content by the two methods. Also, highly positive correlation was found between the results obtained by the direct and extraction method. This indicates the suitability of the direct method for the determination of CLA loaded in β-casein.

CONCLUSION

The direct method offers the advantage of being rapid, simple and direct determination of the entrapped CLA in β-casein nanoparticles without the need solvent extraction of CLA from the particles before spectrophotometric measurement.

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