

Co-encapsulation of fish oil, phytosterol ester and limonene using dairy proteins and soluble corn fibre as wall materials.



Quek S.Y., Chen Q., Wen J., McGillivray D, Zhong F.

School of Chemical Sciences, The University of Auckland, New Zealand (sy.quek@auckland.ac.nz)

INTRODUCTION AND OBJECTIVE

Co-encapsulation of core materials may enhance the bioactivity of individual components (Halwanil et al. 2008). This technique has been widely used in pharmaceutical delivery systems, either in liposome or in capsule form. However, the application of co-encapsulation to deliver more than one bioactive components into food matrix, as we attempted in this work, has been limited in literature.

The intake of plant sterols together with fish oil has been reported to contribute to synergistic effect on cardiovascular health, due to the reduction of cholesterol and the ratio of LDL/HDL (Khandelwal et al. 2009, Micallef & Garg 2008). The intake of both the abovementioned bioactive lipophilic components (BLCs) has also been shown to reduce systemic inflammation in hyperlipidemic individuals (Micallef & Garg 2009). In addition, phytosterols has been found to increase the oxidation stability of lipids (Yasukazu & Etsuo 2003). Therefore, co-encapsulation of fish oil with phytosterols may exhibit promising health benefits. Limonene is a natural flavor which may mask fishy odor, and deliver health benefits including chemopreventive activity against cancers, gastric acid neutralizing effect, supporting normal peristalsis and dissolving cholesterol-containing gallstones (Sun 2007).

The objective of this work was to study the feasibility of applying co-encapsulation to protect fish oil from oxidation and to mask the unpleasant fishy odor by incorporating phytosterol esters (PE) and limonene as core materials together with fish oil. Whey protein isolate (WPI), sodium caseinate (NaCA) and soluble corn fibre (SCF) were used as wall materials. The oxidative stability, *in vitro* digestibility and sensory profiles of the microcapsules were evaluated.

MATERIALS AND METHODS

Materials WPI (ALACENTM 895,) & NaCA (ALANATETM 1800) were from Fonterra NZ Ltd.; SCF (PROMITORTM 70) from Tale & Lyle, Shanghai, China; fish oil (Croda IncromegaTM TG3322) from Nutura NZ; phytosterol esters (CoroWiseTM) and limonene (VALENCIA) from Cargill Inc., USA. Standards for gas chromatography (37 FAMES, internal standards i.e. C13:0 & C23:0), and enzymes (Pepsin, pancreatin and bile salts) were from Sigma-Aldrich, St. Louis, MO, USA.

Methods Microencapsulation by spray drying was performed as described in Chen (2012). Oil-in-water emulsions of good stability were prepared prior to spray drying. The emulsions consisted of either fish oil (FO) or FO mixed with bioactive components (75% FO, 12.5% PE and 12.5% limonene wt. % total oil), milk protein mixture (WPI/NaCA 1:2) or protein/SCF blend (WPI/SCF 1:1) and 70% w/w deionised water. Microencapsulation efficiency (ME) was calculated as (Total extractable oil – Extractable surface oil/Total extractable oil) × 100. The oxidation stability of the microcapsules was evaluated in a storage trial (45 °C, 30 % RH in saturated O₂) for 7 days. Peroxide value (PV), anisidine value (AV) and fatty acid content of the oil phase were determined as well as sensory evaluation and *in vitro* digestibility of the co-encapsulated microcapsules (Chen 2012).

RESULTS AND DISCUSSION

The WPI/SCF system had significantly higher oil recovery and ME ($p < 0.05$) than those with WPI/NaCA (Table 1). The presence of SCF might provide protection for the protein that adsorbed on oil droplets from denaturation by heat, consequently decreased the loss of core materials.

Table 1 : Oil recovery and microencapsulation efficiency of microcapsules

Samples	Oil recovery (%)	ME (%)
FO-WPI/NaCA	95.1 ± 0.7 ^b	97.8 ± 0.1 ^b
FO-WPI/SCF	98.8 ± 0.3 ^a	98.7 ± 0.0 ^a
BLC-WPI/NaCA	91.1 ± 0.6 ^c	97.7 ± 0.1 ^b
BLC-WPI/SCF	98.6 ± 0.6 ^a	98.7 ± 0.0 ^a

Superscripts with different letter in the same column are significantly different ($p < 0.05$) from each other.

The microencapsulated samples had better oxidative stability than the bulk oil as indicated by the PV (Fig. 1) and AV (data not shown, AV had similar trend as PV). Samples having WPI/NaCA as wall material demonstrated relatively lower oxidation than those with WPI/SCF. This could be supported by the lower oxygen permeability of the protein film in our subsequent study (data not shown).

The retention of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the co-encapsulated BLC-microcapsules was higher than samples with FO only. This was especially true in systems containing WPI/NaCA as wall matrix ($p < 0.05$) (Fig. 2). The

results indicated that BLCs and protein matrix could provide better protection for fish oil. This was further supported by sensory evaluation of the microcapsules. The BLC-microcapsules exhibited significantly lower oxidized fishy odors than the FO-microcapsules ($p < 0.05$) after drying and accelerated storage as perceived by a trained sensory panel (Table 2).

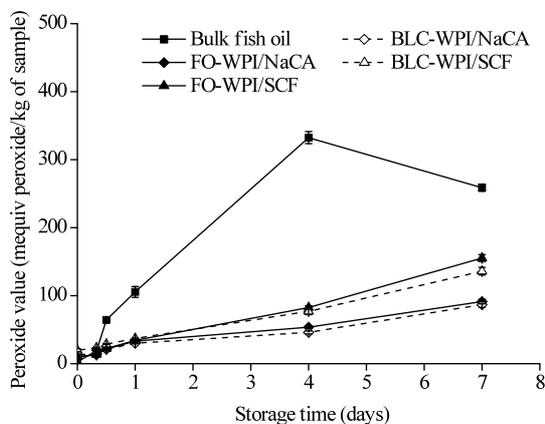


Figure 1 : Changes in the peroxide value (PV) of bulk fish oil, encapsulated fish oil and BLCs microcapsules

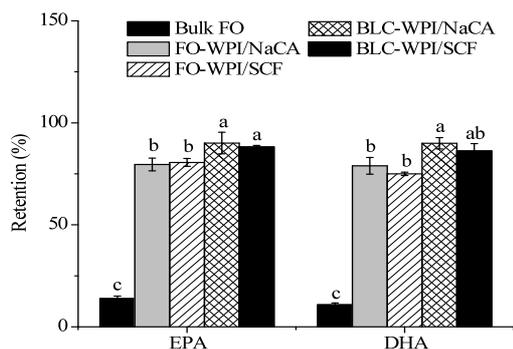


Figure 2 : Change of EPA and DHA in fish oil and microcapsules containing fish oil and BLCs after 7-day-accelerated storage

Table 2 : The intensity of oxidized fishy odor of the microencapsulated fish oil and BLCs at day 0 and after 7-day accelerated storage

Oxidized fish oil odor	Samples			
	FO-WPI/NaCA	FO-WPI/SCF	BLC-WPI/NaCA	BLC-WPI/SCF
Day 0	0.1 ± 0.5	0.2 ± 1.4	Not detected	Not detected
Day 7	2.8 ± 0.5 ^b	3.4 ± 0.5 ^c	1.8 ± 0.6 ^a	2.2 ± 0.7 ^a

In vitro digestion showed that the microcapsules containing WPI/NaCA as wall materials were easier to be lipolysed than those containing WPI/SCF (Fig. 3). The presence of soluble fibers might reduce the digestibility of lipids through different mechanisms

including inhibition of digestive enzyme activities and micelle formation.

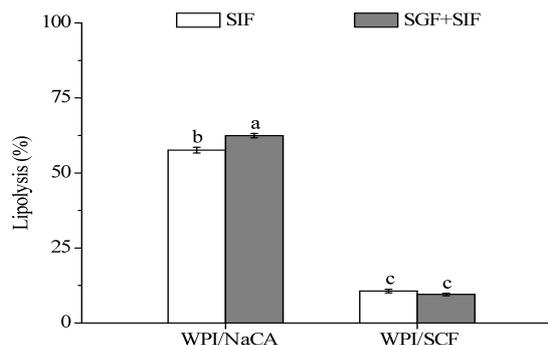


Figure 3: Percentage of lipolysis after exposure of BLC-microcapsules to simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) + SIF

CONCLUSIONS

Co-encapsulation of fish oil with PE and limonene using dairy proteins and SCF could provide better protection from oil phase oxidation during drying and storage. The ME, oxidative stability and digestion profile of the microcapsules could be influenced by the composition of wall materials.

REFERENCES

- Chen Q (2012) *Coencapsulation of fish oil with phytosterol ester and limonene*. PhD thesis, The University of Auckland, New Zealand.
- Halwanil M et al. (2008) *Co-encapsulation of gallium with gentamicin in liposomes enhances antimicrobial activity of gentamicin against Pseudomonas aeruginosa*. Journal of Antimicrobial Chemotherapy, 62, 1291-1297.
- Khandelwal S (2009). *Independent and interactive effects of plant sterols and fish oil n-3 long-chain polyunsaturated fatty acids on the plasma lipid profile of mildly hyperlipidaemic indian adults*. British Journal of Nutrition, 102, 722-732.
- Micallef M & Garg M (2008) *The lipid-lowering effects of phytosterols and (n-3) polyunsaturated fatty acids are synergistic and complementary in hyperlipidemic men and women*. The Journal of Nutrition, 1086-1090.
- Micallef M & Garg M (2009). *Anti-inflammatory and cardioprotective effects of n-3 polyunsaturated fatty acids and plant sterols in hyperlipidemic individuals*. Atherosclerosis, 204, 476-482.
- Sun J (2007) *D-Limonene: Safety and Clinical Applications*. Alternative Medicine Review, 12, 259-264.
- Yasukazu Y & ETSUO N (2003) *Antioxidant effects of phytosterol and its components*. Journal of Nutritional Science and Vitaminology, 49, 277-280.