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EMULSIFYING AND POV CONTROLLING CAPACITY OF WHEY PROTEINS

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ABSTRACT : The emulsifying capacity (EC) of whey proteins (WP) was investigated at different pHs and temperatures in ghee, corn, soya, salad and fish oils, by both visual and electrical conductivity methods. The EC and emulsion stability of heat and pH treated WP decreased in comparison with unheated samples at various pHs. A more stable emulsion from fish oil was formed with WP at a wide range of pHs (2 to 7) than that from the other oils tested. The peroxide value (POV) of the fish oil was controlled with the aid of WP. This study showed that WP may be used as an emulsifier and oxidative stabilizer for fish oils.

Introduction

Whey proteins (WP) are milk proteins remaining in the serum or whey after the precipitation of caseins by chymosin or by acids at pH 4.6 and are composed of β-lactoglobulin (β-Lg), α-lactalbumin (α-La), serum albumins, immunoglobulins and proteose-peptones.

Emulsion is a system where droplets of fat constitute the dispersed phase, water is the continuos phase and protein is the emulsifying agent. Becker[1] reported that the electrical resistance of protein solution or its conductivity, was used to determine the type of emulsion produced.

This method was found to vary with added water, salt concentration, pH, temperature, protein concentration, blending speed and the ratio of oil addition[17]. Thus, a modified method has been widely used by researchers as the indicator of the relative value of meat proteins as emulsifiers [2,3,4,13]. Determination of the EC of WP does not clearly indicate the stability of the emulsion during subsequent processing. There is no actual relationship between EC and emulsion stability, although several techniques are being used to determine emulsion stability by taking samples before to the actual collapse of the emulsion. Swift[18] has proposed that both EC and emulsion stability are needed in determining the emulsifying ability of proteins. Borton et al.[2] used a visual method to determine the time required to de-emulsify as an index of emulsion stability. Carpenter and Saffle[3] have reported that the EC of proteins is related to the change and shape of the protein molecule. Our circular dicroism (CD) study of WP

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(unpublished) showed that EC and emulsion stability were effected by the type of oil, pH and temperature of the protein solution. A more stable emulsion of WP was formed from fish oil than that from the other oils tested. On the other hand, WP formed an unstable emulsion with ghee.

This study was undertaken to investigate the EC of WP at various pHs, and to explore the possibilities of WP as an emulsifier and oxidative stabilizer for the preservation of fish oils.

Materials and Methods

Whey protein concentrate (80% protein, 6% fat, 7% lactose, 3% ash and 4% water) was obtained from Morinaga Milk Industry Co. Fish oil from Nihon Yushi Co. Ltd., salad (soya, corn and rapeseed oil), soya and corn oil was purchased from a local market. Ghee was prepared from local unsalted butter by evaporating the moisture of the butter by heating it at 100℃ for 3 to 4 hours and filtering with cotton cloth to separate the sediments of curd. The fat content of the ghee was within the range of its specification (in India and Nepal) at 99.5%.

Whey protein concentrate was dissolved in deionized water. Its residual fat and sediments were removed by centrifuging for 30 min at 20,000 ×g, to prepare the WP solution. The protein concentration of the WP solution was determined by micro Kjeldahl method and adjusted according to need. The pH of WP was adjusted using standard solutions of HCl and NaOH at 0.1N concentration.

All the chemicals used in this study were obtained from Sigma Chemical Co., USA.

Measurement of emulsifying capacity

Determination of the EC was carried out by keeping a 10g solution of WP in a beaker placed on a supporting stand. A resistance sensing device, stirrer propeller, and oil delivery system were also assembled. The stirrer was immersed in the protein solution and rotated at 2000 rpm, and the oil was delivered at a constant rate of 1.5 ml per sec. The temperature of the oil was maintained at 25℃, except for the ghee (40℃), and the actual process of emulsion formation and collapse was rcorded by electrical resistance measurement using an ATTO electronic recorder, Model No. FR-31. Electrical resistance was applied as the end point criteria to stop the flow of oil at the point of emulsion collapse. The results are expressed by oil (gm)/protein (mg).

Analogous sample supernatants were used to investigate the visual end-point criteria and considered as controls to compare the results with resistance methods. The visual end-point was determined by carefully observing the de-emulsification of the continuous and dispersed phases. Tests for every oils and ghee were completed in triplicate.

Determination of POV

The POV was determined taking 5g of fish oil with and without adding 10% WP concentrate The samples were preserved into conical flasks (200 ml) with air tight covers and kept in dark place at 30℃ for the interval of 0,24 and 48 hrs, and the POV was determined[11].

Determination of odor

The odor concentration of fish oil emulsion with WP was measured using odor concentration meter, Model XP-329, New Cosmos Electric Co. Ltd. Japan. The result was arbitrarily expressed by the reading of the odormeter.

Results

Table 1 contains the condensed results of many experiments, presenting average values for the experiments of EC and emuison srability of WP. The effect of pH on EC with fish, soya, salad oils and ghee is showr· .n Table 1. The lowest value of EC was obtained at pH 5, which is near the pI of WP, regardless of the kind of oil. Whey proteins with pH 2 to 7 and heating at 85℃ for 15 min showed lower EC than unheated samples (Table 1). The decrease in EC of heated samples was thought to be caused by the partial irreversible denaturation of WP by heat and pH. Whey proteins heated at 85℃ for 15 min at pH 5 formed fine precipitation. Only soluble proteins are responsible to show their high ability to emulsify the fats and oils[19].

Table 1 also shows the difference in EC between unheated and heated WP at pH 2 to 7. The largest decreased in EC caused by the heating of WP at 85℃ for 15 min was observed at pH 6 in the case of fish, corn, and soy oils, although it was at pH 4 and pH 3 for salad oil and ghee, respectively. The decrease in EC of WP by heating is thought to be caused by the weaker interaction between heated WP and those of tested oils than that of unheated condition.

Table 1. Difference in emulsifying capacity of unheated and heated whey protein solu-- tions(WP) in selected oils and ghee at pH 2 to 7.

pH of WP	2	3	4	5	6	7
EC of WP	*4.18	3.91	3.00	2.41	3.43	2.80
with fish oil	**4.05	3.37	2.98	2.33	2.42	2.38
	·0.13	0.54	0.02	0.08	1.01	0.42
EC of WP	*4.22	3.86	3.20	3.20	3.48	3.42
with corn oil	**3.66	3.31	3.16	2.50	2.45	2.65
	·0.56	0.55	0.04	0.07	1.39	0.77
EC of WP	*4.18	3.52	3.43	3.29	3.88	3.54
with soy oil	**3.61	3.56	2.65	2.63	2.68	2.93
	·0.57	-0.04	0.78	0.66	1.20	0.61
EC of WP	*4.20	3.80	3.78	3.05	3.11	3.21
with salad oil	**3.92	3.52	2.72	2.95	2.92	3.20
	·0.28	0.28	1.06	0.01	0.09	0.01
EC of WP	*3.92	3.80	3.46	3.07	2.81	3.80
with ghee	**3.50	3.16	3.25	2.90	3.00	3.45
	·0.42	0.64	0.21	0.17	-0.19	0.35

* emulsifying capacity of WP at pH of 2 to 7.
** emulsifying capacity of WP at PH of 2 to 7, heated at 85℃ for 15 min and cooled down to 25℃
· difference in EC between heated and unheated WP at pH of 2 to 7,

Table 2. EC of WP in different oils and ghee at pH (2 to 7) showing maximum emulsion stability at room temperature (25℃)

Oil source	Emulsion stability (hrs.)	Emulsifying capacity oil(g) /protein(mg)	pH
fish oil	300	2.80	7
corn oil	24	3.20	5
soya oil		3.52	3
salad oil (corn, soya and rapeseed oil)	35	3.8 3.8	3
ghee*	0.5	3.8	7

* (emulsion stability of ghee was obsered at 40℃)

The stability of emulsions as well as emulsifying capacity is important to show the functionality of WP. The emulsion stability was studied by visual and electrical conbuctivity methods. The stability of emulsions was mostly dependent on pH of WP solution and the type of oils. Table 2 hows the best conditionfor stability of emulsions of each oil an ghee formed with WP solution. Ghee and fish oil emulsions at pH 7 have higher stability than the emulsions formed in other pHs. Soya, salad and corn oils showed highly stable emulsions with WP solution with pH 3, 3 and 5 respectively. The stability of fish oil emulsion was much higher than any of other oils and ghee.

Figure 1 is a photograph taken after 300 hrs standing of fish oil emulsions with WP at 25℃. Under the same experimental conditions other experimented oils did not satisfy to maintain emulsion stability in comparison with fish oil. It was also shown in Fig. 1 that fish oil formed the most stable emulsion at pH 7 and the least stable at pH 5. In the same time, organoleptic test showed that a smell was lacking in the emulsion of fish oil with WP at pH 7 and 25℃. The odor concentration of fish oil emulsion was 40 which was very near to control (fresh fish oil has odor concentration 35). This effect was considered to be caused by the protection of the conformation of long chain polyunsaturated fatty acids with WP especially the DHA and EPA which are more active to oxidation and production of undesirable flavors. The thiols of WP may exert a anti-oxidant role and decompose the peroxide in the fish oil emulsion.

Since fish oil is the most unstable oil for oxidation among the oils studied, POV was determined after keeping at 30℃ in unlightened condition. The results in Fig. 2 demonstrated that the fish oil oxidation at 30℃ was susceptible and retarded by the application of WP. The results indicated that the POV of fish oil without WP increased more rapidly than that of WP added. Only slight increase in POV was detected in the emulsion with WP after 48 hrs. The effect of WP is thought to be caused due to binding of β-Lg in the long chain of fatty acids in fish oil which acls as an ante-oxidant. Trace amount of lactose with WP might showed a little bit facilitating effect to the process of antioxidation in fish oil. The maillard product found in heated WP may also act an anti-oxidant.

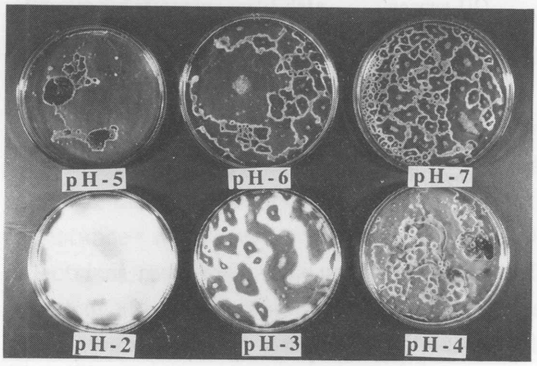

Fig. 1 Whey protein emulsions with fish oil stored at 25℃ over 300hrs. 6mg/ml whey protein solution from pH 2 to 7 was separately emulsified up to saturation with fish oil at 25℃ and kept for stability observation by visual and electcical conductuity methods. pH 7 was found most stable.

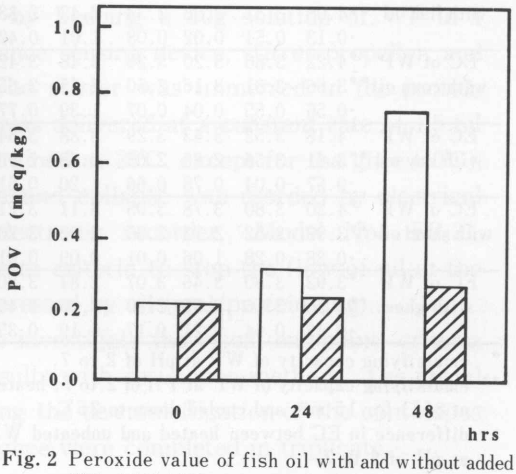

Fig. 2 Peroxide value of fish oil with and without added WP. 10% WP was mixed with 5g of fish oil, kept at 30℃ over 0, 24 and 48 hrs in darkness, and POV was analyzed. 100% fish oil was taken as control.

□ POV of solely fish oil,

▨ POV of fish oil with adding WP

Discussion

Among the sensitive factors that alter the functional properties of proteins are pH and temperature. Alfa lactalbumin can promote complex formation with β-Lg through disulfide reactions at elevated temperatures[21]. There was a detectable difference in the conformation of β-Lg heated at 72℃ [5] and similar result for α-La was obtained by CD method[9]. Whey proteins showed different emulsifying potential with corn, soya, salad, fish oils and ghee. Especially fish oil was well emulsified with WP. The decrease in EC at pH 5, near the pI of WP, was thought to be caused by the conformational changes of WP. The ability of protein solutions to form self-supporting gels depends on a favorable balance of attractive and repulsive forces between protein molecules[6]. The increase in EC at pH below 5 is thought to be caused by the excess amount of repulsive charges in WP solutions.

In earlier studies on EC of β-Lg was explained that, in the range of pH 3-8 does not undergo a change in its EC, although it does undergo conformtional changes[22]. Some authors such as Haydon et al. and Nielsen et al.[8,10] demonstrated the contrary, that is, the emulsion stability could be higher at the pI. Emulsifying activity of BSA is pH dependent and sharply decreases as the protein conformation changes[8,12,22]. However, it is obvious that the proteins do not bear net charge at pI and the charge-based contribution tends to be minimum and permits proteins to coagulate in compact form with increased surface area and enhances to form cohesive films.

Perhaps, the relatively high stability of emulsion at pI is thought to be caused due to the coverage of higher surface area by protein and the structure that forms more cohesive films that enhances stability. The lacking of odor in fish oil emulsion was observed. Beta lactoglobulin in its native state, binds aromatic hydrophobes at low molar ration[15], retinol[7], detergents[20] and long chain fatty acids[14,16] . The WP used in the present study was already heated by pasteurization of raw milk before cheese making. The masking effect, however, of WP could be still available, and was useful advantage of WP.

In conclusion, the EC and emulsion stability of WP was strongly effected by pH, although EC was significantly varied with the type of oils. Butter oil and ghee are not same milk products and their production technology also varies, due to the big size of ghee crystals and solidifying property at room temperature (32℃) might lower the emulsifying stability. Emulsifying capacity of WP heated at 85℃ for 15 min with pH 2 to 7 was decreased with comparison of unheated WP samples. To study the EC of heated whey protein, temperature was chosen at 85℃ which is near the mid range of functionalization temperature of WP. Fish oil and WP demonstrated more stable eulsions at pH 7 than those of other tested oils. The POV of fish oil was controlled at higher temperature (30℃) with adding 10% WP concentrate in unlightened condition. However, further studies are necessary to determine the mechanisms of interaction of WP and fish oil by which the oxidation is controlled.

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要　約

コーン油，大豆油，サラダ油，魚油およびギー(無塩バターから水分を除去したものでインドやネパールで室温で長期保存できる食品として利用している）を用い，様々な条件下でホエータンパク質の乳化能を調べた。乳化能は，混合物の外観と電気伝導度によって判定し，さらに酸化抑制効果を調査するために過酸化物価（POV）を測定した。

ホエータンパク質を，熱処理すると乳化能や乳化安定性が減少した。広い pH 域（2～7）で，ホエータンパク質は実験に供した油脂類の中で魚油に対して最も安定なエマルションを形成した。また，ホエータンパク質は魚油の POV を抑制する効果にも優れていることが示された。この研究結果は，魚油の乳化剤あるいは抗酸化剤としてホエータンパク質が利用できることを示している。