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Evaluation of Fish Gelatin and Sodium Alginate Blend as Gelling Agents for Pudding Containing Virgin Coconut Oil

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ABSTRACT

Virgin coconut oil (VCO) is known for its functional properties but it is yet to be widely developed into food products. Lipid and gelling agents are crucial ingredients for the rheological characteristics of milk puddings. This study aimed to explore the potential of blends of fish gelatin and sodium alginate as gelling agents and a delivery system for VCO. A total of 15 pudding formulations were generated from a mixture design approach to determine the optimized proportions of VCO (6-13%), fish gelatin (2-6%), sodium alginate (0.15-0.75%), and water (65-75%). All 15 pudding formulations exhibited strong elastic characteristic with their *G*' (storage modulus) values higher than the *G*" (loss modulus). Formulations with high contents of gelatin (6%) and sodium alginate (0.28-0.75%) exhibited strong gel characteristics (tan $\delta < 0.1$). The optimized formulation consisted of 10.68% VCO, 3.41% fish gelatin, 0.59% sodium alginate, and 68.33% water, with desirability of 0.874 against the viscoelastic properties and firmness of a commercial pudding. A significant increase was observed in firmness and free fatty acid (FFA) value of the optimized pudding from 3 weeks onwards, over the weekly evaluation of 4 weeks storage at 4 ± 1°C. The sensory assessment showed that rancidity of pudding was not significantly detected by panels throughout the 4 weeks of storage period.

Keywords: Fish gelatin, mixture design, pudding, sodium alginate, virgin coconut oil

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INTRODUCTION

Virgin coconut oil (VCO) is gaining increasing popularity in both the scientific field and the public as a functional food supplement. VCO is mechanically obtained from the kernel of the coconut under mild temperature or without heat, without undergoing chemical refining, bleaching

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or deodorizing (Marina et al., 2009). VCO is considered to have numerous beneficial health effects attributed to its mode of processing that prevents the loss of its beneficial components such as vitamin E and polyphenols (Nevin & Rajamohan, 2008). Studies also showed that VCO possesses antibacterial, antiviral, antinociceptive, anti-inflammatory and lipid lowering effect in blood (Nevin & Rajamohan, 2008; Zakaria et al., 2011). VCO can be consumed directly, but its strong coconut scent has limited its palatability, which makes the incorporation of VCO into a food system an attractive approach. To date, the availability of food products for delivering VCO remains limited in the market.

Milk puddings that are widely consumed by children and elderly people can be effective in delivering food components with health benefits. The consumer acceptability on puddings depends on their gel characteristics, particularly rheological and textural properties that are defined by the type and the content of the fat and gelling agents as well as their interactions (Saha & Bhattacharya, 2010; Toker et al., 2013). The use of gelling agent blends in food products is able to bring synergetic effect to achieve a desirable texture (Li & Nie, 2016; Toker et al., 2013). Among gelling agents, gelatin finds maximum application in food products, such as yoghurt products, low-fat spread and sugar confectionery, owing to its low melting temperature and slow-setting gelation behaviour (Saha & Bhattacharya, 2010). Lately, fish gelatin is gaining prominence to replace the commercial mammalian gelatin and to increase the utilization of fish by-products (skins and bones) discarded from the fish processing industry as the gelatin source (Karim & Bhat, 2009). Meanwhile, alginate derived from brown seaweeds is a preferred gelling agent for restructured foods, puddings and desserts due to its fast-setting gelation (McHugh, 2003; Saha & Bhattacharya, 2010). It is therefore possible that the blends of gelatin and alginate would be potential in modulating the gelation strength of a food product. So far, there is no study conducted on food product development with the use of gelatin-alginate blends.

Therefore, the objective of this study was to develop a pudding formulation by optimizing the content of VCO, fish gelatin, sodium alginate and water using mixture design methodology, for achieving the targeted rheological and textural properties of a commercial pudding. The optimized pudding formulation was further evaluated for its texture, hydrolytic rancidity and sensory stability during storage for 4 weeks at refrigeration temperature.

MATERIALS AND METHODS

Materials

The VCO was purchased from a local VCO producer in Kudat, Malaysia. Fish gelatin and sodium alginate derived from seaweed was purchased from a food ingredient supplier (Klang, Malaysia). All other ingredients, inclusive of sugar, skimmed milk powder and soy lecithin were purchased from a local bakery ingredients supplier. Commercial pudding product was purchased from a local market.

Preparation of Puddings

Gelatin was added with water and stirred for 5 min at 50 ± 2°C. Lecithin (0.15%, w/w) was dissolved completely in VCO and then added gradually to the gelatin solution under stirring for 10 min at 50 ± 2°C to form emulsion. In a separate preparation, skimmed milk powder (7%, w/w), sugar (9.85%, w/w) and sodium alginate were mixed and dissolved in water, stirred continuously for 10 min at 85 ± 2°C, and then cooled to 50 ± 2°C. Then, the emulsion was added gently into the mixture and stirred for another 20 min at 50 ± 2°C. The puddings were poured into plastic containers, cooled to room temperature (23 ± 2°C) and stored in a refrigerator (4 ± 1°C) for 1 h prior to the analyses.

Experimental Design and Statistical Analysis

D-optimal mixture design of Design Expert software v. 7.0.0 (State-Ease Inc., Minneapolis, MN, USA) was employed in formulation study. The ranges of ingredients obtained from the preliminary study were used, which were the VCO (6-13%, w/w) (*A*), fish gelatin (2-6%, w/w) (*B*), and sodium alginate (0.15-0.75%, w/w) (*C*), and water (65-75%, w/w) (*D*) to make up to a constant amount (83%). A total of 15 pudding formulations was generated, consisting 12 formulations of different contents with 3 formulations were replicates (Table 1). The rheological behavior of puddings was evaluated by measuring the viscoelastic properties and firmness, and the values (*Y*) were fitted to three equation models of the linear, quadratic and cubic models (Equations (1)-(3)). The statistical significance of each equation was analyzed through analysis of variance (ANOVA) at $p<0.05$ using the same software. The optimized content of these ingredients in formulating pudding was generated using the numerical optimization technique of the same software by setting the viscoelastic properties and firmness measured on a commercial pudding as targeted values. Validation of the optimized pudding formulation was performed by comparing the experimental values to the predicted values, where comparisons were carried out by LSD t-test ($p<0.05$).

$$Y = b_1A + b_2B + b_3C + b_4D \tag{1}$$

$$Y = b_1A + b_2B + b_3C + b_4D + b_{12}AB + b_{13}AC + b_{14}AD + b_{23}BC + b_{24}BD + b_{34}CD \tag{2}$$

$$Y = b_1A + b_2B + b_3C + b_4D + b_{12}AB + b_{13}AC + b_{14}AD + b_{23}BC + b_{24}BD + b_{34}CD + b_{123}ABC + b_{124}ABD + b_{134}ACD + b_{234}BCD \tag{3}$$

where *Y* is the estimated responses (viscoelastic properties and firmness); *b* is the constant coefficients for linear and non-linear (interaction) term.

Viscoelastic Properties and Firmness Measurement of Puddings

Viscoelastic properties of pudding samples were determined using an AR 1500EX rheometer (TA Instruments, Delaware, USA) with a serrated parallel plate geometry of 20 mm and a gap of 1000 μm. The oscillation

Table 1
Viscoelastic properties and firmness of pudding formulations in a mixture design

Formulation	VCO (%)	Fish Gelatin (%)	Sodium alginate (%)	Water (%)	*G'* (Pa)	*G''* (Pa)	*G** (Pa)	tan δ	Firmness (N)
1	6.00	3.83	0.15	73.02	747.9182 ± 56.1185	48.0755 ± 5.8239	749.4773 ± 56.3693	0.0642 ± 0.0030	15.4300 ± 1.4142
2	12.86	4.99	0.15	65.00	4941.7273 ± 81.6387	605.8409 ± 56.3049	4979.4091 ± 88.1312	0.1236 ± 0.0097	21.6750 ± 1.6900
3	7.00	2.29	0.75	72.95	1352.7728 ± 193.9401	156.4955 ± 12.9529	1361.9546 ± 194.0686	0.1168 ± 0.0074	4.0050 ± 0.0212
4	9.47	4.56	0.45	68.52	4117.2273 ± 356.3175	443.7228 ± 43.1271	4141.6364 ± 349.5679	0.1090 ± 0.0199	13.0000 ± 0.2263
5	10.50	6.00	0.28	66.22	4191.5909 ± 235.5951	399.3637 ± 11.3522	4210.6818 ± 235.3380	0.0960 ± 0.0028	24.0700 ± 1.0607
6	12.87	4.39	0.74	65.00	40036.8182 ± 1931.6872	24510.4546 ± 3671.1698	47132.2727 ± 148.4924	0.6345 ± 0.0839	15.4700 ± 0.2121
7	7.06	6.00	0.75	69.19	9589.8728 ± 251.2672	973.6727 ± 18.8219	9701.0000 ± 164.8202	0.1011 ± 0.0003	19.5350 ± 0.0071
8	11.81	2.00	0.28	68.90	275.9227 ± 25.6680	24.4114 ± 1.5421	277.0409 ± 25.7066	0.0878 ± 0.0022	8.2100 ± 0.6081
9	12.87	4.39	0.74	65.00	56305.4896 ± 6023.2147	29331.9805 ± 2235.5136	63594.7437 ± 6232.5446	0.5437 ± 0.0433	16.2600 ± 0.8910
10	13.00	3.41	0.35	66.23	4266.5909 ± 433.8421	477.6228 ± 60.3934	4264.4091 ± 478.5827	0.1119 ± 0.0029	13.0450 ± 0.0778
11	6.94	6.00	0.17	69.89	3391.1364 ± 532.1942	226.7818 ± 64.6553	3398.6818 ± 535.4084	0.0662 ± 0.0086	21.4000 ± 0.3394
12	9.59	2.00	0.15	71.26	259.1091 ± 5.5797	44.1005 ± 0.7643	262.9591 ± 5.3804	0.1647 ± 0.0071	5.2450 ± 0.5869
13	6.00	2.00	0.36	74.64	266.6864 ± 50.0825	45.1627 ± 8.3567	270.4818 ± 50.7703	0.1713 ± 0.0016	1.8250 ± 0.1202
14	7.06	6.00	0.75	69.19	7820.5455 ± 1359.8288	690.9091 ± 143.2727	7851.0000 ± 1403.1570	0.0883 ± 0.0028	21.2350 ± 0.7990
15	7.00	2.29	0.75	72.95	1502.7273 ± 213.9320	192.4500 ± 25.3337	1514.9546 ± 215.4104	0.1287 ± 0.0014	3.7100 ± 0.0141

frequency sweeps were performed over the range of 0.1-10 Hz at 0.2 Pa. Measurements were carried out at 4°C and a constant strain of 1%. The storage modulus (G'), loss modulus (G''), complex modulus (G^*), and loss tangent ($\tan \delta = G''/G'$) were measured as a function of frequency and calculated using the TA rheometer Data Analysis software (Version V. 4.20, TA Instruments Inc.) (Alamprese & Mariotti, 2011; Toker et al., 2013). Firmness of pudding samples was measured using a TA.XTplus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a diameter plate of 35 mm connected to a load cell of 490.33 N. Penetration tests were performed to 30% of the initial height of the sample and the load (N) recorded was used as result (Alamprese & Mariotti, 2011).

Storage Stability of Optimized Pudding Formulation

Storage stability of pudding was assessed weekly over a period of 4 weeks at 4 ± 1°C. Texture stability of pudding in terms of firmness was measured using a TA.XTplus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) according to the method by Alamprese and Mariotti (2011). Hydrolytic rancidity was evaluated through determination of FFA values (%, as lauric acid) according to AOAC 940.28 (Association of Official Analytical Chemists [AOAC], 1999). For to FFA analysis, pudding was melted completely at 40°C under continuous stirring and centrifuged at 689 x *g* for 15 min. The upper oil layer was collected for FFA determination. Sensory stability in terms of rancidity was assessed by 30 semi-trained panelists from the Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Malaysia. Panelists were trained and standardized with a rancid oil sample (Villarino et al., 2007). Paired comparison test was used to detect rancidity in the stored puddings with freshly produced pudding used as control. At least 20 judgements out of the 30 panels were required for the pudding sample to be rancid significantly at 5% level of significance (Larmond, 1977). All tests for storage stability were carried out in triplicate. Experimental results were reported as mean value with standard deviation and significant differences at the probability level of $p<0.05$ was determined using one-way ANOVA with Tukey test (SPSS v. 21.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Model Fitting

Table 1 shows the results of viscoelastic properties and firmness measured for each pudding formulation from mixture design study. Results of ANOVA suggested the quadratic and linear model were the best for the viscoelastic properties and firmness, respectively (data not shown). The VCO-fish gelatin, VCO-sodium alginate, VCO-water, fish gelatin-sodium alginate, fish gelatin-water, sodium alginate-water quadratic model terms had antagonistic effect on the viscoelastic properties of the pudding formulations. For the firmness of the puddings, the VCO and fish gelatin had the positive effect, but the sodium alginate and water had the negative effect. Fish gelatin had the highest positive effect, while sodium alginate had the highest negative effect on the firmness of the pudding, which is in agreement with the antagonistic effect of alginate reported by Toker et al. (2013).

Table 2 shows the quadratic and linear models for the data of viscoelastic properties and firmness, respectively. Their *R*-squared values of higher than 0.74, and *p*-values of lack of fit of higher than 0.05 (data not shown) indicated the adequacy of the models in predicting the variation of the results (Henika, 1982; Sarteshnizi et al., 2015).

Viscoelastic Properties and Firmness of Puddings

As shown in Table 1, all puddings exhibited strong elastic characteristic as their *G*' values were higher than the *G*" values (Saha & Bhattacharya, 2010). The *G** values were close to the *G*' values and the tan δ values were less than 1, indicated that all pudding formulations were highly elastic (Lim & Narsimhan, 2006; Toker et al., 2013). A few pudding formulations exhibited strong gel characteristic as reflected by tan δ values less than 0.1 (Saha & Bhattacharya, 2010). The blends of high contents of fish gelatin (6%) and sodium alginate (0.28-0.75%) produced puddings with strong gel characteristics (formulations 5, 7, 14). Strong gel strength was also exhibited for puddings (formulations 1, 11) consisted of low content of sodium alginate (0.15-0.17%) when added with a lower amount of VCO. An addition of lipid can increase the *G*', *G*" and tan δ values, attributed to the formation of protein-lipid complexes (Tolstoguzov, 2003). Thus, the low tan δ values were probably attributed to the lower VCO content. Further test found that puddings with strong gel characteristic were firmer (formulations 5, 7, 11, 14).

Table 2
Predicted models for the viscoelastic properties and firmness of pudding

Parameter	Predicted models	R^2
G'	844226.7076 *A* + 6499591.4760 *B* + 565244793.8239 *C* + 91884.8132 *D* - 4167580.2181 *AB* - 490130017.7576 *AC* - 1564627.5020 *AD* - 559354960.7603 *BC* - 7726608.7507 *BD* - 580510174.5598 *CD*	0.9560
G"	276854.5715 *A* + 4249945.2456 *B* + 352915852.8623 *C* + 57278.6239 *D* - 2514666.8587 *AB* - 303496662.5973 *AC* - 680844.8931 *AD* - 356302456.5589 *BC* - 5070906.9855 *BD* - 362593041.5131 *CD*	0.9819
*G**	907448.6305 *A* + 7693781.1451 *B* + 661989380.4897 *C* + 107537.8709 *D* - 4884583.4044 *AB* - 573277851.3198 *AC* - 1735571.9504 *AD* - 657297063.5143 *BC* - 9148713.7094 *BD* - 679910507.7974 *CD*	0.9640
tan *δ*	-0.0811 *A* + 102.0512 *B* + 6321.2461 *C* + 1.4474 *D* - 31.5686 *AB* - 5441.8332 *AC* - 7.6245 *AD* - 6213.7732 *BC* - 126.5370 *BD* - 6504.8091 *CD*	0.9720
Firmness	34.9614 *A* + 360.2353 *B* - 387.5188 *C* - 6.7183 *D*	0.9635

A: VCO, *B*: fish gelatin, *C*: sodium alginate, *D*: water

Optimization of Pudding Formulation

Optimization of pudding formulation was performed by setting the tan δ value of 0.2 and firmness value of 11 N (obtained for a commercial pudding) as targeted values. Pudding formulation comprised 10.68% VCO, 3.41% fish gelatin, 0.59% sodium alginate and 68.33% water were selected as optimized formulation based on the highest desirability of 0.874, with predicted tan δ value of 0.2 and firmness value of 11 N. The optimized formulation was validated through experiments and was found to have no significant difference (p>0.05) between the predicted and experimental values of tan δ (0.1954 ± 0.0048) and firmness (11.93 ± 2.72 N).

Storage Stability of Puddings

Figure 1 shows the results obtained from storage stability study on the optimized pudding formulation over 4 weeks at 4 ± 1°C. Firmness of pudding increased significantly (p<0.05) from 3 weeks onwards (Figure 1 (a)). According to Alexa et al., (2010), a more solid structure of spreads developed over storage period was likely attributed to the formation of fat crystal network during slow post-crystallization processes. This could possibly explain that the increase in firmness of pudding in this study was ascribed to the presence of VCO. Likewise, FFA of pudding increased significantly (p<0.05) from 3 and 4 weeks onwards (Figure 1 (b)). The increase in FFA values suggested the hydrolytic degradation of

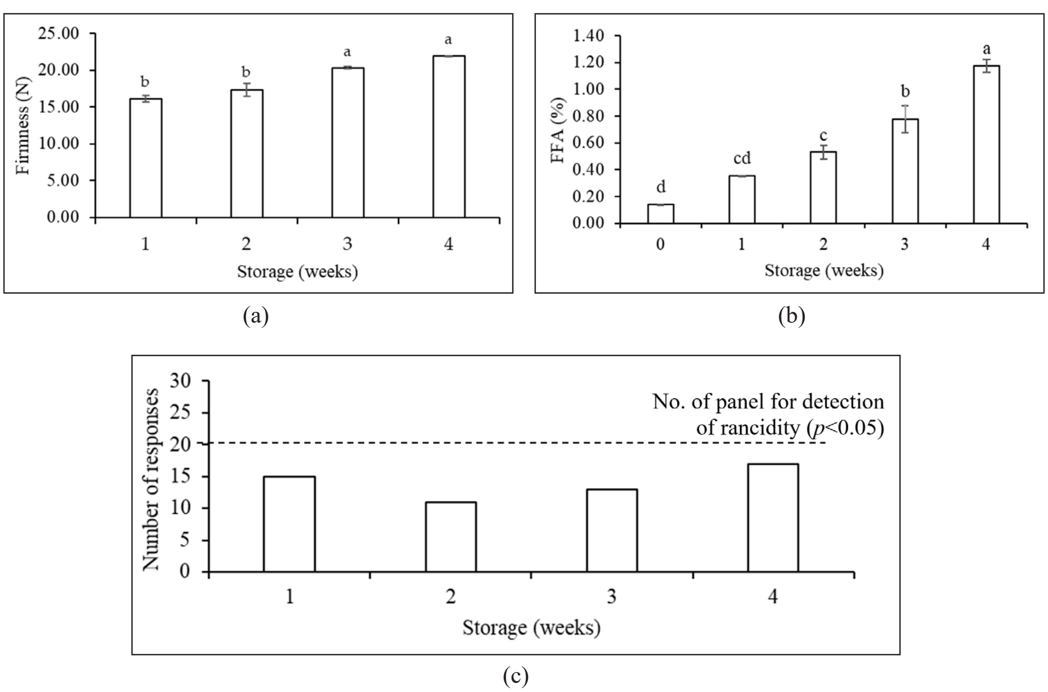

Figure 1. Results of storage stability of pudding over 4 weeks at 4 ± 1°C: firmness (a), FFA value (b), and count of panel who detected rancidity of pudding (c). Different letters indicate significant difference (p<0.05)

VCO in pudding. According to Eke-Ejiofor and Beleya (2015), the presence of the lipase activity or other hydrolytic action on lipid is responsible for the hydrolytic degradation that leads to an increase in FFA values of salad creams over storage period. The production of FFA can lead to rancidity that results in off-flavours of products (Talbot, 2016). Sensory assessment performed on pudding in this study found that rancid taste was not significant (p>0.05) when evaluated weekly (Figure 1 (c)), although significant increase in FFA values was observed from 3 weeks onwards. Results generally revealed that this pudding was stable during storage.

CONCLUSION

Optimized pudding formulation containing 10.68% VCO, 3.41% fish gelatin, 0.59% sodium alginate and 68.33% water was successfully obtained using mixture design and experimentally validated. Blending of high fish gelatin content with sodium alginate produced puddings with increased in the viscoelasticity and firmness. The firmness and FFA values of the puddings increased significantly from 3 weeks onwards, and the rancid taste was not statistically significant over 4 weeks of storage period at refrigeration temperature.

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