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Modeling and Optimization of Physicochemical and Organoleptical Properties and *Lactobacillus acidophilus* Viability in Ultrafiltrated Synbiotic Cheese, Containing Microbial Transglutaminase Enzyme, Whey and Inulin

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Abstract

The growing demand of consumers for healthy foods has created a great incentive for the advancement of new food products around the world. Functional foods, particularly synbiotic products, are one of these products. In this research, the effects of microbial transglutaminase enzyme (MTG, 0-1 units per gram of milk protein), demineralized whey powder (DWP) solution containing 34% DWP (0-16%) and inulin (0-2%) on the physicochemical, sensorial and microbial properties of Iranian white ultrafiltrated synbiotic cheese was investigated using the response surface method (RSM). For cheese production, *Lactobacillus acidophilus* LA5 was used as probiotic and inulin and DWP solution were used as prebiotics. The results showed that by increasing of MTG concentration, moisture content of cheeses increased significantly ($P<0.05$), but acidity and other physicochemical properties and sensory attributes did not change noticeably. By increasing DWP substitution with ultrafiltrate, fat and protein values ($P<0.01$) and all the sensory attributes ($P<0.05$) significantly decreased but acidity did not change remarkably. Furthermore, with increasing inulin, acidity, color and appearance, odor and flavor ($P<0.05$) increased and moisture content ($P<0.01$) decreased significantly. By increasing of MTG concentration, the number of probiotic bacteria reduced significantly but addition of DWP solution ($P<0.05$) and inulin ($P>0.05$) had adverse effect and enhanced it. The optimization results showed that by using 0.43 U/g protein of MTG, 8.24% DWP solution and 0.71% of inulin, an Iranian white synbiotic ultrafiltrated cheese with appropriate physicochemical and sensory properties could be produced. The optimized cheese had adequate total acceptability (7.76 score) and high probiotic count (6.96 logcfu/g).

Keywords: Inulin, *Lactobacillus acidophilus*, RSM, Synbiotic cheese, Transglutaminase enzyme

Introduction

The growing demand of consumers for healthy foods has created a great incentive for the advancement of new food products around the world. Functional foods, particularly synbiotic

products, are one of these products. The term synbiotic means a product containing both prebiotics and probiotic bacteria. Prebiotics are non-digestible food constituents that promote the growth of probiotic bacteria (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). Probiotics are a group of live microorganisms which in adequate amounts ($>10^6$) impart beneficial effects on the host's health (Donkor, Nilmini, Stolic, Vasiljevic, & Shah, 2007). Cheese is an appropriate carrier for probiotics owing to lower acidity, high-buffering capacity and nutritious compounds particularly milk fat and protein. The commercial probiotic bacteria are mostly members of the *Lactobacillus* genus such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei* and *Lactobacillus plantarum* (Tripathi & Giri, 2014). Ultrafiltrated-Iranian white cheese is a common type of cheese produced in Iran and mostly consumed as a breakfast cheese. Therefore, ultrafiltrated-Iranian white cheese is a good vehicle for probiotics and could be considered as a synbiotic product. It has been shown that inulin and whey proteins have the potential to be as a fat replacer, prebiotic agent and texture modifier. Besides, the cheese structure and its rheology can be improved via enzymatic treatment of milk proteins by transglutaminase. Microbial transglutaminase (MTG) catalysis covalent cross-linking of proteins such as caseins and causes intra- and intermolecular linkages. However, this new casein network may affect the growth rate and viability of probiotics. The effect of prebiotics such as inulin on synbiotic cheeses (Araújo, de Carvalho, Leandro, Furtado, & de Moraes, 2010; Jirsaraei, Pourahmad, & Fadaei, 2017) and effect of MTG on the quality of low-fat ultrafiltrated cheeses (Danesh, Jooyandeh, & Goudarzi, 2017a, 2017b) have been recently investigated. However, simultaneous impact of inulin and whey on quality of ultrafiltrated-Iranian white synbiotic cheese using microbial transglutaminase have not been studied yet. Therefore, the objective of this study was enzymatic incorporation of whey proteins into the formulation of UF-Feta cheese by MTG and utilization of inulin to obtain a low-fat synbiotic cheese with acceptable texture, sensory properties and a desirable viability of probiotic bacteria.

Material and methods

Three-variable-three-level Box-Behnken design-response surface methodology (BBD-RSM) with five replications at central point was used to optimize the variable parameters includes MTG, DWP and Inulin concentrations. All the cheeses produced with 34% total solids and all the analysis were performed after 2 months storage period.

Results and discussion

The enzymatic treatment with MTG significantly increased moisture content of cheeses. Covalent cross-linkages formed by MTG created a firmer casein network and thereby enhanced water holding capacity of the cheeses and resulted in the lower cheese syneresis. Sensory evaluation of cheese samples showed that treatment with MTG significantly ($P<0.05$) increased cheese hardness owing to intra- and intermolecular protein linkages. At the higher levels of MTG (near 1 U/g protein), the cheese texture score drastically decreased. As DWP substitution levels increased, fat and protein values of cheeses significantly ($P<0.01$) decreased but moisture and acidity did not change noticeably. An important interaction between MTG levels and DWP substitutions were observed, i.e. at the lower DWP substitution levels (near 0% substitution), as the MTG concentration increased, the cheese protein decreased probably due to the higher cheese moisture (Fig. 1). The lower fat and protein contents in the DWP substituted cheeses were due to the lower of these constituents in DWP in comparison with ultrafiltrate. This caused weaker and improper three-dimensional casein network in the cheese curd. By increasing DWP substitution with ultrafiltrate up to 8%, all the sensory attributes were improved while at the higher levels of substitution, all the sensory scores drastically decreased ($P<0.05$).

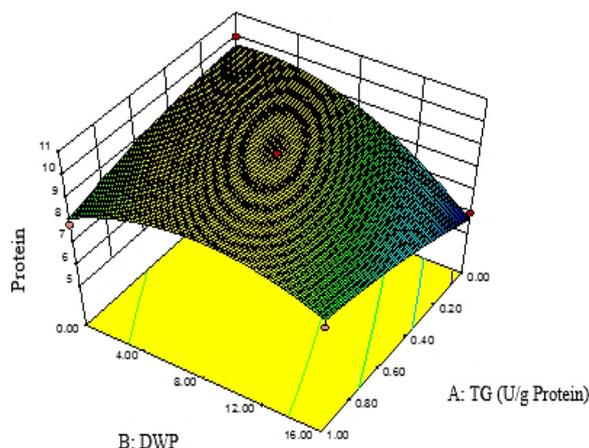


Fig. 1. Response surface plots for interaction effects of MTG concentrations and DWP substitution levels on protein content of reduced-fat ultrafiltrated synbiotic cheese

Moreover, the addition of the inulin had positive effect on all the sensory scores and enhanced the cheese acidity but reduced the cheese moisture. The sample contained 1 U/g protein MTG and 0% inulin had the highest moisture content. Besides, the synbiotic cheese containing 2% inulin and 0% DWP had the best color and appearance and the cheese sample having 1 U/g protein MTG and 2% DWP had the lowest texture score. The addition of inulin and DWP substitution had positive effect on the viability of probiotic bacteria, but MTG treatment had adverse effect and reduced its viable count. In general, the determination coefficient (R^2) more than 0.9 for all the parameters suggested that all of the models were valid, implying that more than 90% of the variation could be explained by the fitted models. The adjusted determination coefficient (R^2_{Adj}) was used to evaluate the correlation between the experimental values and predicted values, and the outcome 0.81-0.93 for physicochemical and sensory attributes suggested that the correlation was significant. Also, “lack of fit” for all the assessed parameters were not significant. The CV for all the parameters ranged from 1.54 to 5.21 for the physicochemical characteristics and from 5.54 to 9.84 for the sensory properties which defined a good reliability of the experimental values.

Conclusion

The optimization results shown that by using 0.43 U/g protein of MTG, 8.24% DWP solution and 0.71% of inulin, an ultrafiltrated-Iranian white synbiotic cheese with appropriate physicochemical and sensory properties could be produced. The optimized cheese after two months of storage may have adequate total acceptability (7.76 score) and high probiotic count (6.96 log CFU/g). Moreover, under these conditions the predicted values for protein, fat, moisture and titratable acidity were 10.21%, 14.10%, 66.29% and 92.55 °D. Based on the results, manufacturing of this optimized low-fat synbiotic cheese is recommended as this functional food can promote the community health.

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