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Selection and optimization of salt replacer, flavour enhancer and bitter blocker for manufacturing low sodium Cheddar cheese using response surface methodology

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ABSTRACT

Salt reduction in cheese is a challenge since it leads to defects in body, off-flavour development and bitter after-taste owing to its key role in changes during ripening. Cheddar cheese was chosen due to its high consumption in industrialized countries together with high concentration of sodium (approximately 6 g kg^{-1} cheese) which may result in hypertension and cardiovascular disease. Present study envisaged to reduce sodium content of Cheddar cheese by using potassium based salt replacer in combination with flavour enhancers and bitter blockers to mask the off-flavours and bitterness recognized in Cheddar cheese containing potassium salts. Various commercial products and combinations were tried in solutions; Saloni K, hydrolyzed vegetable protein and adenosine-5′-monophosphate (AMP) gave the highest saltiness and lowest bitterness scores. To optimize their content in Cheddar cheese, response surface methodology was used; and responses were flavour, body & texture, colour & appearance, saltiness and bitterness. Low sodium cheese with 75% sodium chloride substitution, 2 g L^{-1} hydrolyzed vegetable protein and 300 mg L^{-1} AMP gave best results without sensory loss.

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1. Introduction

Sodium chloride (NaCl) is a vital ingredient of almost all varieties of cheeses and serves various functions such as providing flavour, syneresis, and controlled microbial growth etc (Guinee & Fox, 2004). However, excess dietary sodium causes an increase in blood pressure levels, hypertension and cardiovascular diseases with age (Cruz et al., 2011). Cheddar cheese contains approximately 6 g kg^{-1} sodium (USDA, 2011) and its increased consumption may result in concomitant increase in dietary sodium intake above recommended level of 5 g NaCl per day for an adult (WHO, 2012) and thus attempts have been undertaken to reduce sodium levels in Cheddar cheese. Partial replacement of NaCl with potassium chloride (KCl) has been the most preferred method of reducing sodium content in Cheddar cheese. However, bitter and metallic flavour and soft and pasty body are the most common defects encountered (Cruz et al., 2011). Though, Grummer, Bobowski, Karalus, Vickers, and Schoenfuss (2013) reported the possibility of altering the source of KCl to eliminate these defects. Further, to overcome the problems of off-flavour development, strategies such as adding flavour enhancer and bitter blocker could be utilized.

Research attempts on taste–taste interaction have concluded that if stimuli are strong then suppression of flavour perception may occur. Contrary to this, weak or near threshold stimuli results in enhancement of overall flavour perception (Keast & Breslin, 2002). Lawrence et al. (2011) attempted to use odour–taste interaction in lipo-proteic matrices used as solid food model systems. Lipo-proteic matrices were prepared and flavoured with comtè cheese flavour and it was suggested that salt-associated odours such as comté cheese can enhance saltiness in complex solid-food matrices containing a low amount of NaCl. Similar approach could be utilized in monitoring the effect of taste–taste interaction on saltiness enhancement in low sodium Cheddar cheese. Flavour enhancer can be used to produce the effect of taste–taste interaction on low sodium Cheddar cheese made by partial replacement of NaCl with KCl. Flavour enhancers are responsible for their umami, brothy, and savory taste which opens the opportunity to produce low sodium products with high saltiness intensity and can mask bitter flavour (Desmond, 2006). Flavour enhancers such as monosodium glutamate (MSG), hydrolyzed vegetable protein, yeast extract, disodium

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inosinate, and disodium guanylate have been tried in combination with KCl in Cheddar cheese (Grummer et al., 2013); meat and meat products (McGough, Sato, Rankin, & Sindelar, 2012); soups and salad dressing (Kremer, Mojet, & Shimojo, 2009) to overcome the flavour issues of KCl. Drake et al., 2007 suggested that umami taste plays role in flavour profile of Cheddar cheese due to the presence of glutamic acid. They also reported that umami intensity increases with ripening of Cheddar cheese and flavour attributes such as sulphur and brothy are perceived. Bitter blocker is a compound which blocks the activation of the gustducin in taste receptor cells and thereby prevents taste nerve simulation (McGregor, 2004). Most excessively used among them is adenosine-5′-monophosphate (AMP) which improves the taste of mixtures such as NaCl and KCl (McGregor, 2004). These bitter blockers are reported to eliminate the alkaline and bitter off-flavours of KCl solutions (Desmond, 2006). Glycine may also function in blocking bitterness as 40% of NaCl replacement was achieved in fermented sausages by mixture of glycine and potassium lactate with no reported bitterness (Gou, Guerrero, Gelabert, & Arnau, 1996). Meat industry has exposed the ability of bitter blockers in bitterness blocking potency however till date there is no finding on the effect of bitter blocking potency of these compounds in cheese.

Therefore, the present investigation aimed (1) to select the formulation among various combinations of salt replacer, flavour enhancer and bitter blocker having higher saltiness and lower bitterness intensity in aqueous solution and (2) to examine the effect of adding the selected formulation on sensory attributes of low sodium Cheddar cheese using response surface methodology.

2. Materials and methods

2.1. Raw materials

Cow's fresh milk was procured from the Experimental Dairy, National Dairy Research Institute, Karnal (Haryana), India. Microbial rennet (Meito) commercially produced in granular form from *Mucor pusillus* var. *Lindt* was procured from Meito Sangyo Co., Ltd., Tokyo, Japan. Commercial-grade fine NaCl and KCl salt was obtained from M/S Tata Chemicals, Mumbai, India and Polypharma Pvt. Ltd., Mumbai, India respectively. Saloni and Saloni K salts were procured from NMS Pharma, Gujarat, India. Saloni and Saloni K are vegetable origin potassium rich salt replacers manufactured from the plant of *Salicorniabranchiata* and *Kappaphycusalvarezii*, respectively. Hydrolyzed vegetable protein (soy based) and yeast extract powder were procured from Chaitanya Agro-Bio Tech Pvt. Ltd., Maharashtra, India. Inosine-5′ monophosphate (IMP), AMP, Glycine and Lysine were procured from Sigma Aldrich Co. LLC. All the chemicals and reagents used for chemical analysis were of AR grade.

2.2. Selection of formulation

2.2.1. Preparation of salt solutions

Three types each of salt replacer (Saloni, Saloni K and KCl), flavour enhancer (hydrolyzed vegetable protein, yeast extract and IMP) and bitter blocker (Lysine, AMP and Glycine) were selected (Table 1) for screening of one from each on the basis of their saltiness enhancement properties. Solutions were prepared in distilled water. NaCl solution (2 g L^{-1}) was prepared as control. For preparation of low sodium solution, 50% of NaCl was replaced with potassium salt such that weight of NaCl and KCl ratio in the solution become 1:1. These were then added with 5 g L^{-1} flavour enhancer and 100 mg L^{-1} bitter blocker. All the solutions were prepared 24 h prior to testing and were stored in glass bottles at 4 °C until used. Solutions were subjected to sensory evaluation at 21 °C.

2.2.2. Sensory panel and experimental procedure

Sensory panel of ten trained judges took part in evaluation of triplicate samples in three sessions of 30 min each. All participants reported normal ability for taste perception. Judges were selected on the basis of their threshold for salty and bitter taste. They were requested not to eat and smoke one hour prior to evaluation sessions. Panelists were placed in separate booths and solutions were provided at 21 °C. Twenty millilitres of each solution was presented in a 50 mL glass beaker. In 30 min session, nine samples were provided with the interval of 90 s between the samples. This process was repeated in triplicate on the same day with the interval of 30 min between the sessions. For each sample, panelists were asked to rate saltiness and bitterness of the solutions on a linear scale of 0–10 where higher scores signifies higher intensity (desirable for saltiness) and lower scores were provided for lesser intensity (desirable for bitterness). Cleansing of mouth with distilled water between each sample was also done. Taste induced saltiness enhancement (TISE) was calculated for the selected combination by calculating the difference of saltiness intensity of solution containing selected flavour enhancer, bitter blocker and salt replacer with that of the intensity of solution containing only selected salt replacer.

2.3. Modelling of responses

A central composite face-centered response surface methodology (RSM) design with three independent variables was generated (Table 3). There were six replicates at the centre point for a total of 30 experimental trials (Table 4). Analysis was performed using Design-Expert® Version 8 software (Stat-Ease Inc. Minneapolis, MN, USA). The independent variables were: NaCl replacement (X_1), hydrolyzed vegetable protein (X_2) and AMP (X_3). Responses for the study were sensory attributes of Cheddar cheese *i.e.* flavour (Y_1), body & texture (Y_2), colour & appearance (Y_3), saltiness (Y_4) and bitterness (Y_5). To fit the data for each response to quadratic model, multiple linear regression analysis was performed. The accuracy of the concerned model was checked through lack-of fit tests and other adequacy measures such as R^2, adjusted R^2_{adj} and PRESS (Table 5). The quadratic equation used for each response variable is given in Eq. (1):

$$Y = \beta_0 + \beta_1 * X_1 + \beta_2 * X_2 + \beta_3 * X_3 + \beta_{12} * X_1 * X_2 + \beta_{13} * X_1 * X_3 + \beta_{23} * X_2 * X_3 + \beta_{11} * X_1^2 + \beta_{22} * X_2^2 + \beta_{33} * X_3^2 + \varepsilon \quad (1)$$

where, β_0 is the intercept, β_1, β_2, and β_3 are first order coefficients; β_{12}, β_{13} and β_{23} are the interaction (cross point) coefficients; and β_{11}, β_{22} and β_{33} are second order coefficients, and ε is random error. To show the effect of independent variables on responses, three dimensional plots were generated for each response variable.

Table 1
Salt replacers, flavour enhancers and bitter blockers used in preparing the solutions for saltiness and bitterness rating with the aim to select one from each category and subsequently to use in Cheddar cheese manufacturing.

Salt replacers	Flavour enhancers	Bitter blockers
Saloni	Hydrolyzed Vegetable Protein (HVP)	Lysine
Saloni K	Yeast Extract (YE)	Adenosine 5′-Monophosphate (AMP)
Potassium Chloride (KCl)	Ionosine 5′-Monophosphate (IMP)	Glycine

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