SUBSTITUTION OF MILK ALLERGEN INGREDIENT BY BLOOD PLASMA POWDER IN CUSTARD WITH DIFFERENT SWEETENER

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Abstract

Animal blood is a by-product, which can be utilized in a value-adding way instead wasting. Allergen substitution is a good possibility especially for a substance that is difficult to substitute, such as milk. Blood plasma is a fluid with high protein content without blood (iron) taste and colour, so it is similar to milk in several ways. In case of investigation of substitution of milk, it is advisable to investigate the substitution of sugar as well because a lot of consumers, which exclude milk from their diet, find the glycaemic index and energy content of foods important. Colour, pH and rheological attributes were measured. According to the results the used protein source as well as sweetener significantly determine the colour, pH and texture of the final product. However, colour and pH are easy to change by other components and the effect of substitution of milk and sugar on rheological attributes might not be able to detect without instrumental analysis.

Keywords: allergen substitution, animal blood, animal by-product, product development, sustainability

Introduction

According to the biological definition, milk is a liquid, which is produced by mammals, and which is serving the newborns as food and contains all nutrients newborns need for development (Spreer, 2017). Thus, it is very hard to substitute milk as an ingredient in foods. But unfortunately, milk is an allergen food and food ingredient as well (Goldman et al., 1963).

Milk contains average 3.2% protein, in which main fractions are caseins (82.62%: 30.85% α_{S1}-casein, 7.45% α_{S2}-casein, 44.33% β+κ-casein in proteins) and whey proteins (17.38% in proteins) (Ceballos et al., 2009). Bovine blood plasma contains 7.9% protein, in which main fractions are albumins (41.77% in proteins), immune-globulins, α- and β-globulins (53.17% in proteins) and fibrinogen (5.06% in proteins) (Halliday, 1975; Howell & Lawrie, 1983). The comparison of amino acid composition of these two materials can be seen in table 1.

Table 1.: Amino acid composition of bovine blood plasma and cow milk in mass percent per total amino acid content (Based on Duarte et al., 1999; Ceballos et al., 2009; WHO, 2007)

Amino acid	Bovine blood plasma [mg/100 amino acid]	Cow milk [mg/100 amino acid]
Val	6.73	5.24
Ile	3.35	4.54
Leu	9.34	9.44
Thr	6.6	4.11
Cys	3.36	0.82
Met	0.86	2.48
Tyr	4.87	5.67
Phe	5.16	4.73
His	9.94	3.3
Lys	4.18	8.96
Try	7.47	no data
Asp	1.18	7.6
Ser	9.8	5.24
Glu	6.67	19.66
Pro	4.74	8.99
Gly	3.39	1.75
Ala	5	3.41
Arg	3.3	4.06

Utilization of blood is important, but not only for allergen substitution but also for sustainability. 3-5% of an animal's whole weight is blood, which can be produced during bloodletting after slaughtering (Halliday, 1975). This high amount of blood is mostly annihilated instead of value-adding further-processing because too few blood-based food products are widespread and popular in Europe. Implementing technical conditions of collecting blood for human consumption, which are required according to the Regulation 853/2004/EC, are expensive for smaller slaughterhouses. So, annihilation is necessary if the blood product cannot be sold according to the Directive 91/271/EEC and Commission Directive 98/15/EC (chemical oxygen demand maximum: 125 mg/L). Chemical oxygen demand of blood is about 400 g/L and the biological oxygen demand of blood is about 200 g/L (Hsieh & Ofori, 2011). Thus, producing functional foods with blood or blood fractions for a special consumer group is a good opportunity. There were already investigations in the topic of substituting egg (Caldironi & Ockerman, 1982; Raeker & Johnson, 1995).

Custard is a perfect test matrix for investigation of substituting milk by blood plasma, because it is simple to handle and has only a few ingredients. Nearly all foods are colloid systems. Custard dessert consist of two phases: 1.) a continuous aqueous phase containing

starch and/or carrageenan and 2.) a dispersed phase of oil. The role of proteins is to stabilize the dispersed phase (René et al., 2003). Thus, firstly the effect of blood plasma and milk proteins can be considered through texture properties. First factor of this research, which was investigated, was the protein depending on the raw material: 1.) milk and 2.) blood plasma. First aim of this study was investigating the effect of milk and blood plasma on sensory and techno-functional attributes measured by instrumental methods.

The type of sweetener material was the second factor in this research. Sucrose represents one level and sugar alcohols (xylitol and erythritol) represent another level. Xylitol is a polyalcohol, which is marked with 'E 967' according to the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives, and it has 2.4 kcal/g energy content, 7 glycaemic index and 0.4 sweetening value. (Sweetening value of sucrose is 1.) It does not cause tooth decay, but it has a laxative effect in excessive amount (Melaia & Hamalainen, 1977). Erythritol, which is marked with 'E 968', has 0.2 kcal/g energy content, 0 glycaemic index and 0.6-0.8 sweetening value. It extends the self-life of bakery products, but there is a loss of the sweetening value above 160 °C (De Cock & Bechert, 2002). Second aim of this study was to investigate the effect of milk and blood plasma on attributes of sponge cake with different sweetener, because a lot of consumers, which exclude milk from their diet, find the glycaemic index and energy content of foods important.

Materials and methods

Materials

The aim was to substitute allergenic milk protein by blood plasma protein. At the same time fat content of milk (2.8%) has been substituted as well with sunflower oil, which has a naturalistic flavour. Overplus water content of milk was replaced by drinking water. Blood plasma was made from an easily handleable plasma powder by diluting it to the same protein content as the milk had. Thus, the protein content of custard made with blood plasma powder, was closely the same as with milk. Raw material specifications and food nutrition database (USDA, 2018) were used for calculating the recipe. Plasma powder 70B (Sonac Burgum B.V., Netherlands) was used. The used sweetener mix was made of erythritol:xylitol in 55:45 ratio, which has an equal sweetening effect as the common crystal sugar (sucrose). Recipes are shown in table 2.

Table 2. Ingredients' mass [g] in recipes of different investigated products

Ingredients	Vanilla custard with milk and sugar	Vanilla custard with milk and sweeteners	Vanilla custard with blood plasma and sugar	Vanilla custard with blood plasma and sweeteners
Modified corn starch (g)	5	5	10	-
Vanilla aroma (g)	1	1	-	10
Milk (g)	100	100	-	-
Blood plasma powder (g)	-	-	4.3	4.3
Water (g)	-	-	92.9	92.9
Sunflower oil (g)	-	-	2.8	2.8
Crystal sugar (g)	10	-	10	-
Sweetener mix (g)	-	10	-	10

Methods

Colour measurement

Minolta CR-400 (Konica Minolta, INC., Japan) chroma meter was used for the reflection colour measurement. The measurement is based on the fact that any colour can be generated by the mixture of three ones defined by the light wavelength. The ratio of these three different wavelength lights are plotted in a coordinate system called CIELAB colour space. The colour coordinates can be coded by numbers making colours analysable.

The instrument was calibrated with a standard white etalon. Each sample was measured three times. Measured attributes were the followings: redness/greenness (a*), yellowness/blueness (b*) and brightness (L*). Total colour difference was calculated according to the following equation:

Equation (1) $\Delta E^*_{ab} = \sqrt[2]{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$, where differences are calculated between means of different sample groups: $\Delta L^* = L^*_1 - L^*_2$, $\Delta a^* = a^*_1 - a^*_2$, $\Delta b^* = b^*_1 - b^*_2$ (Dawson & Acton, 2018).

pH measurement

Voltcraft PHT-02 ATC pH stick (Voltcraft®, Germany) pH meter was used for pH measurement. The principle of pH sticks operation is based on electronic differentiation between a referent electrode with a stable value and a pH-sensitive electrode in a fluid with any standard redox potential. Sample pH is calculated from the potential difference according to a

linear correlation. The device was calibrated before each measurement series with two standard buffers. Each sample were measured three times.

Rheological measurement

Anton-Paar Physica MCR 91 (Anton-Paar GmbH, Germany) viscometer was used for rheological measurements. The behaviour of samples (especially theoretical viscosity) was measured under variable speed shear stresses with concentric cylinders (CC27) and Couette type method. 2 × 31 data were collected during one measurement run. The RPM of the inner cylinder varied between 1 and 1000 min^{-1}. The outcome of the measurement was a flow curve, to which a model was fitted. This model can define the rheological behaviour of the samples and the rheological parameters can be calculated following the calibration of the model. The flow behaviour of all samples could be described by the Herschel-Bulkley model that considers the following parameters: shear stress (τ), theoretical yield point (τ_0), deformation speed (γ), consistency index (C) and power of law index (p). A new shear rate was calculated from these parameters and this new shear rate validates the compliance of the model. The determination coefficient (R^2) that represents the explained variance rate indicated a highly significant model with its value over 0.99 in case of each sample. Herschel-Bulkley model can be described by the following equation:

Equation (2) $\tau = \tau_0 + C \times \gamma^p$ (Mezger, 2006).

Statistical analysis

Measurement results were evaluated by IBM SPSS statistic v25 (IBM Corp., Armonk, NY) and Microsoft Excel 365 version: 2010 (build: 13328.20356) software. To detect the effect of protein ingredient and sweeteren on rheological parameters, multivariate analysis of variance (MANOVA) was carried out, that can compare the means of different sample groups of related variables. According to Levene's test, the homogeneity of variances was slightly violated ($p<0.05$). The normality of residuals was checked by Shapiro-Wilk test ($p>0.05$). The value of the unexplained variance rate (Wilks's lambda) was evaluated. The homogeneous groups were separated by Tukey post hoc test.

Results and discussion

Colour of different sample groups was similar but distinguishable. Difference was clearly visible to the naked eye. Means of colour parameters are shown in table 3 and total colour difference is shown in table 4.

Table 3. Means of colour parameters (* – redness-greenness colour parameter [-], b* – yellowness-blueness colour parameter [-], L* – brightness colour parameter [-]) of different measured sample groups

Protein source	**Sweetener**	**L***	**a***	**b***
Blood plasma	Sugar	60.25	-0.68	9.61
Blood plasma	Sweetener	45.08	-1.06	6.22
Milk	Sugar	80.13	-4.13	4.15
Milk	Sweetener	79.62	-3.71	6.1

Table 4. Total colour differences (ΔE^* – total colour difference [-]) of different measured sample groups (Darker red colour marks higher difference.)

	Vanilla custard with milk and sugar	Vanilla custard with milk and sweeteners	Vanilla custard with blood plasma and sugar	Vanilla custard with blood plasma and sweeteners
Vanilla custard with milk and sugar	0,00	2,06	20,90	35,25
Vanilla custard with milk and sweeteners	2,06	0,00	19,92	34,64
Vanilla custard with blood plasma and sugar	20,90	19,92	0,00	15,55
Vanilla custard with blood plasma and sweeteners	35,25	34,64	15,55	0,00

Value of pH may have an effect on the texture. So, pH was considered during the evaluation of results. Values of different sample groups were significantly different. This have been caused by the more alkaline attribute of plasma proteins and sugar alcohols than milk proteins and sucrose. Table 5. shows pH values.

Table 5. Means of pH value [-] of different measured sample groups

Protein source	Sweetener	pH
Blood plasma	Sugar	7.88
Blood plasma	Sweetener	8.34
Milk	Sugar	6.33
Milk	Sweetener	6.37

All custard samples were measured two times during the rheological measurement. Thereby, the clotted custard status and the stirred custard status could be investigated as well. The overall MANOVA result was highly significant for protein source, sweetener as well as the two-way interaction of these two factors (Wilks' Lambda: 0.001; 0.001; 0.004 all with $p<0.001$) in case of clotted custard. In case of the second measurement cycle, so in case of stirred custard, the overall MANOVA result was also highly significant for protein source, sweetener as well as the two-way interaction of these two factors (Wilks' Lambda: 0.002; 0.009; 0.007 all with $p<0.001$). In case of stirred custard values of Wilks' Lambda were slightly higher but these indicate a strong effect of factors, too. Means of rheological parameters are shown in table 6.

Table 6. Means of rheological parameters (τ^0 – theoretical yield point [Pa], C – consistency index [Pa s^p], p – and power of law index [-]) of different sample groups

Protein source	Sweetener	τ_0 (Pa)	C (Pa s^p)	p
Blood plasma	Sugar	8.81	4.78	0.52
Blood plasma	Sweetener	18.55	9.07	0.92
Milk	Sugar	3.43	1.68	0.64
Milk	Sweetener	5.22	0.97	0.7

Conclusion

Based on this research the allergenic milk can be substituted by non-allergenic blood plasma in simple food products like custard, but it causes a significant change in sensory attributes. The used protein source as well as sweetener determine the colour, pH and texture of the final product. The caused colour change is clearly visible to the naked eye, but each sample was nearly white and another flavouring and/or colouring matter can mask this change. Texture of different sample groups was different as well, but it could be detected only by instrumental method. A sensory test might not be able to detect it based on our observation.

There is an important suggestion in case of substituting milk protein by blood plasma protein: the used plasma concentrate or plasma powder should have reduced salt content because salt content of blood is high and it is concentrated in the plasma fraction. It causes great flavour change.

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