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Substitution of milk allergen ingredient by blood plasma powder in custard with different sweeteners

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ABSTRACT

Animal blood is a by-product, which can be utilized in a value-adding way instead of being wasted. 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. While investigating the substitution of milk, it is advisable to investigate the substitution of sugar as well because a lot of consumers who exclude milk from their diet find the glycaemic index and energy content of foods important. The investigated model food is a simple, homogeneous matrix: vanilla custard with milk and with and without sugar and vanilla custard with blood plasma and with and without sugar. Colour, pH and rheological attributes of custard sample groups 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 with other components (food colours, acidity regulators) and the effect of milk and sugar substitution on rheological attributes might not be possible to detect without instrumental analysis.

KEYWORDS

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

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INTRODUCTION

Allergen substitution and fight against global obesity are in the focus of media and literature.

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 allergenic food and food ingredient as well (Goldman et al., 1963).

Milk contains on average 3.2% protein, whose main fractions are caseins (82.65%: 30.80% α_{S1}-casein, 7.50% α_{S2}-casein, 44.35% β+κ-casein in proteins) and whey proteins (17.35% in proteins) (Ceballos et al., 2009). Bovine blood plasma contains 7.9% protein, whose 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 and Lawrie, 1983). It is interesting to compare the amino acid composition of these different protein sources to observe their nutritional value. The comparison of the amino acid composition of these two materials can be seen in Table 1. Amino acid composition of milk is more balanced for the human body. It is interesting that the ratio of amino acids which contain sulphur is the opposite in the two different protein sources.

The utilization of blood is important, not only for allergen substitution but also for sustainability. Three-five percent 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 the technical conditions of

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

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

collecting blood for human consumption, which are required according to the Regulation 853/2004/EC, is 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^{-1}). Chemical oxygen demand of blood is about 400 g L^{-1} and the biological oxygen demand of blood is about 200 g L^{-1} (Ofori and Hsieh, 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 and Ockerman, 1982; Raeker and Johnson, 1995).

Custard is a perfect test matrix for the 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 consists 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 (Wijk et al., 2003). Thus, firstly the effect of blood plasma and milk proteins can be considered through texture properties. The first factor of this research which was investigated was the protein depending on the raw material: 1.) milk and 2.) blood plasma.

A lot of consumers who exclude milk from their diet find the glycaemic index and energy content of foods important. Because of this, the type of sweetener material was the second factor in this research. In this research, sucrose and sugar alcohols (xylitol and erythritol) were investigated, because these are the most widely used sweeteners in custards. 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^{-1} 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 amounts (Melaja and Hamalainen, 1977). Erythritol is marked with 'E 968', has 0.2 kcal g^{-1} energy content, 0 glycaemic index and 0.6–0.8 sweetening value. It extends the shelf-life of bakery products, but there is a loss of the sweetening value above 160 °C (De Cock and Bechert, 2002). There is a popular mixture of erythritol and xylitol in 55:45 ratio, which is easy to use, because its sweetening effect is equal to that of common crystal sugar (sucrose).

The aim of this study was to investigate the effect of milk and blood plasma in the presence of different sweeteners (sugar and sugar alcohols) on instrumentally measured sensory attributes like colour and texture as well as techno-functional attributes measured by instrumental methods.

MATERIALS AND METHODS

Materials

To substitute allergenic milk protein by blood plasma protein, an adequate and homogeneous sample matrix had to be found: this was the custard. At the same time, fat content of milk (2.8%) was also substituted, by sunflower oil, which does not have an unusual flavour, for developing a similar sample matrix in the case of each examined protein source. Overplus water content of milk was replaced by drinking water. Blood plasma was made from an easy-to-handle plasma powder by diluting it to the same protein content that the milk had. Thus, the protein content of custard made with blood plasma powder was closely matching that of milk. Raw material specifications and food nutrition database (USDA, 2018) were used for calculating the recipe.

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	5	5
Milk with 2.8% fat content (g)	100	100	–	–
Vanilla aroma (g)	1	1	1	1
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

Plasma powder 70B (Sonac Burgum B.V., Netherlands) was used. The used sweetener mix was made of erythritol:xylitol in 55:45 ratio (Gorky és Zentai Kft., Hungary). Modified corn starch was used for texture developing. Recipes are shown in Table 2.

Samples were produced according to the following procedure: Dry powdered ingredients were mixed well. Then dry ingredients were mixed with the milk or water while being heated. Fluids were heated to the gelatinization temperature, then to the boiling temperature, while stirring continuously. Samples were boiled for 1 min.

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 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 following: redness/greenness (a^*), yellowness/blueness (b^*) and brightness (L^*). Total colour difference was calculated according to the following equation:

$$\Delta E^*_{ab} = \sqrt[2]{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}, \tag{1}$$

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 and 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, Austria) viscometer was used for rheological measurements. The behaviour of samples (apparent viscosity – shear rate function) was measured under variable shear stress with concentric cylinders (CC27) and Couette type method, in which the sample holder cylinder is standing, and the inner cylinder is rotating. Samples were tempered to 25 °C. Two × thirty-one data were collected during one measurement run. The RPM of the inner cylinder varied between 1 and 1,000 min^{-1}. The outcome of the measurement was a flow curve, to which a model was fitted. Each sample group was measured six time. This model can define the rheological behaviour of the samples. The flow behaviour of all samples could be approximated 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 exponent (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 the case of each sample. Solver extension of Microsoft Excel 365 version: 2010 (build: 13328.20356) software was used for fitting and verification of the model. Herschel-Bulkley model can be described by the following equation (Mezger, 2006):

$$\tau = \tau_0 + C \times \gamma^p. \quad (2)$$

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 sweeteners 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. Lightness and redness-greenness of these milk custards were similar to results from other studies (Salami et al., 2019; Térrega et al., 2004), but results of blood plasma custard samples were different.

Value of pH may generally have an effect on the texture because of the relation between the water holding capacity of proteins and distance from the isoelectric point. 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. The pH results of milk

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 ± 0.77	−0.68 ± 0.14	9.61 ± 0.20
Blood plasma	Sweetener	45.08 ± 0.62	−1.06 ± 0.27	6.22 ± 0.43
Milk	Sugar	80.13 ± 1.10	−4.13 ± 0.11	4.15 ± 0.17
Milk	Sweetener	79.62 ± 1.11	−3.71 ± 0.37	6.1 ± 0.38

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 ± 0.26	20.90 ± 0.51	35.25 ± 0.33
Vanilla custard with milk and sweeteners	2.06 ± 0.26	0.00	19.92 ± 0.50	34.64 ± 0.41
Vanilla custard with blood plasma and sugar	20.90 ± 0.51	19.92 ± 0.50	0.00	15.55 ± 0.20
Vanilla custard with blood plasma and sweeteners	35.25 ± 0.33	34.64 ± 0.41	15.55 ± 0.20	0.00

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

Protein source	Sweetener	pH
Blood plasma	Sugar	7.88 ± 0.02
Blood plasma	Sweetener	8.34 ± 0.03
Milk	Sugar	6.33 ± 0.02
Milk	Sweetener	6.37 ± 0.02

custards were similar to the literature (Bassen et al., 1989; Kebede and Ashenafi. 2010; Park et al., 2017).

Rheological behaviour of all custard sample groups was measured in two different ways. The clotted texture and the skin on the surface were broken during first measurement. Then samples from each sample group were stirred and measured again. 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 the case of clotted custard. In the case of the second measurement cycle, i.e., in the 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 the 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. Texture of different sample groups was different, but a sensory test might not be able to detect it based on our observation (small nominal differences). Rheological parameter results of milk custards and the

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

Protein source	Sweetener	τ_0 (Pa)	C (Pa s^p)	p (–)
Blood plasma	Sugar	8.96 ± 1.82	4.78 ± 0.29	0.52 ± 0.02
Blood plasma	Sweetener	18.01 ± 3.23	8.68 ± 0.86	0.52 ± 0.02
Milk	Sugar	3.38 ± 0.71	1.68 ± 0.14	0.58 ± 0.07
Milk	Sweetener	5.11 ± 1.66	0.97 ± 0.24	0.61 ± 0.10

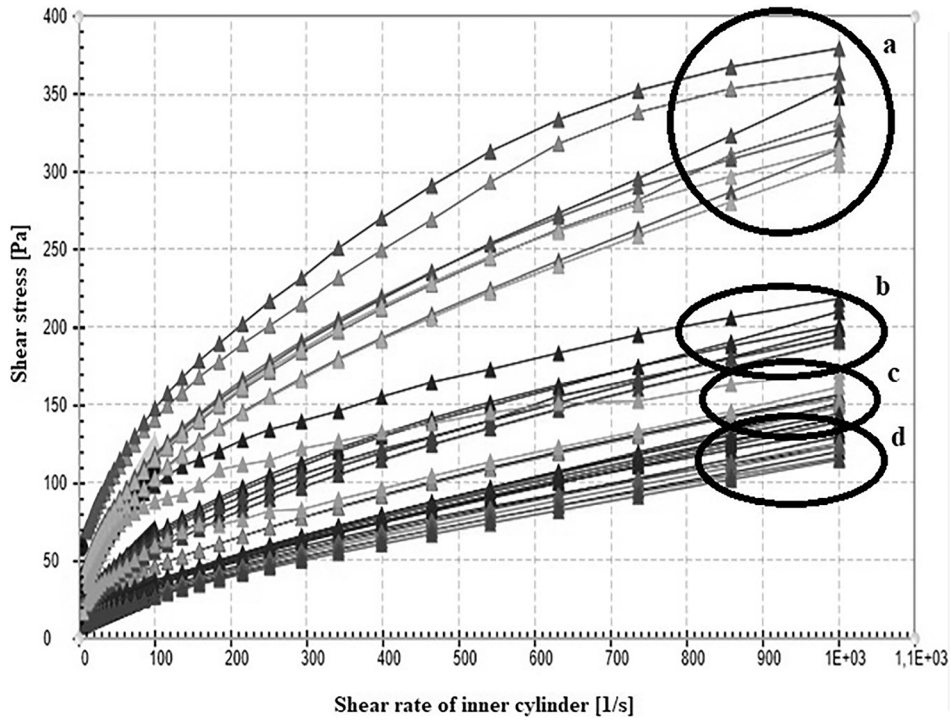

Fig. 1. Some flow curves of investigated sample groups (a – custards with blood plasma and sugar alcohols, b – custards with blood plasma and sugar, c – custards with milk and sugar, d – custards with milk and sugar alcohols) created by Anton Paar RheoCompass software

course of flow curves were similar to results from the literature (González-Tomás et al., 2008; Vélez-Ruiz et al., 2005, 2006). In the case of blood plasma custards, the course of flow curves was similar but the theoretical yield point and the consistency index were a multiple of results of milk custards (Fig. 1).

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 instrumentally measured 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. There is an important suggestion in the 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 considerable flavour change.

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