EGG ALLERGEN INGREDIENT SUBSTITUTION BY BLOOD PLASMA POWDER IN SPONGE CAKE

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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 an obvious possibility because many properties of blood plasma are similar to egg white. Techno-functional and sensory attributes (water activity, moisture content, colour and texture related properties) were measured by instrumental methods. The allergenic egg powder can be substituted by non-allergenic blood plasma powder in sponge cakes, but the change in the ingredient has an effect on hardness and tolerating compressive stress until the breaking. In case of water activity and moisture content, sponge cakes with blood plasma were desirable like sponge cakes with egg.

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

Introduction

Animal blood and separated blood fractions are valuable resources, which are mostly wasted as a hazardous waste (Csurka et al., 2020). If blood is utilized with value-added further processing, it causes environmental, economic, and nutritional benefits as well as can be a tool of sustainability (Ofori & Hsieh, 2014). Consumers also prefer foods from sustainable systems (Floros et al., 2010). Proteins, like blood plasma proteins, which were not used for human consumption so far, will have an important role in the near future because the absolute and relative overpopulation and approaching protein deficiency (Godfray et al., 2010).

Egg is an allergenic, essential food ingredient in bakery products because of its solubility, heat coagulation, foaming, and emulsification properties (Shepherd & Yoel, 1976). It is hard to substitute egg white in bakery products, but blood plasma may be a good alternative for egg allergic consumers.

Animal blood plasma not only holds opportunities for the meat industry, but it is already used in bakery products: fresh, frozen and spray-dried plasma has a similar effect in texture and appearance of cakes like egg white in similar form and amount of (Lee et al., 1991; Johnson et al., 1979). Plasma proteins denature at lower temperatures than major egg white protein (ovalbumin). γ-globulin is the most heat-stable and fibrinogen is the most heat-sensitive protein

in the blood plasma. The foam stability of plasma is similar to egg white, but egg white foam is more stable. Plasma fractions, serum albumin, fibrinogen and α-globulin have a good foaming capacity and stability. In egg white, globulins are the only protein fraction with good foaming properties. Plasma and plasma proteins are better emulsifiers than egg white and egg white proteins (Raeker & Johnson, 1995). However, according to different researches, plasma is still better than other binders like meat powders, gelatine, wheat gluten, isolated soy protein and combination of sodium alginate and calcium carbonate (Lu & Chen, 1999) and all the blood proteins (globins, serum albumin, haemoglobin) are better emulsifiers than egg (ovalbumin) (Caldironi & Ockerman, 1982).

The protein content of bovine blood plasma is about 7.9%, in which the immunoglobulins represent 4.2%, albumins 3.3% and fibrinogen 0.4% (Howell & Lawrie, 1983). According to another research, plasma proteins contain globular proteins (about 60% albumins and 40% globulins) and around 3-4% fibrinogen (Putnam, 2012). Proteins are concentrated in all dried, powdered product. The composition of proteins in case of blood plasma powder are about 50% albumin, 15% α-globulin, 15% ß-globulin and 15% γ-globulin (Makara et al., 2016). The composition of blood plasma naturally depends on the animal species and the parameters of the separation technology. There is difference between the plasma of different animal species (Lynch et al., 2017).

Table 1. Essential amino acid, cysteine and arginine content of different blood fractions and isolates as well as myosin of meat (for comparison) in mass percent per total protein content (Based on Ockerman & Hansen, 1988; Sorapukdee & Narunatsopanon, 2017; Gorbatov, 1998; Makara et al., 2016; Attia et al., 2020; Wang et al., 1997)

Amino acid	**Whole porcine blood**	**Whole bovine blood**	**Blood plasma fraction**	**RBC fraction**	**Fibrin**	**Hemoglobin**	**Serum globulin**	**Serum albumin**	**Whole egg**	**Egg white**	**Egg yolk**	**Myosin of meat**
Tryptophan	no data	1.5	1.2	1.59	3.5	2	2.8	0.7	1.03	1.01	1.37	0.8
Methionine	0.723	2.4	0.6	0.75	2.6	1.2	1	0.8	2.54	2.61	2.34	3.4
Lysine	8.604	9.7	6.1	7.99	9	10.6	6.3	12.8	6.87	6.15	7.88	10.3

Valin	7.244	8.7	4.8	8.65	3.9	11	9.7	5.9	5.35	4.87	5.41	2.6
Threonine	3.624	4.8	1.2	2.94	7.9	6	7.4	5.8	1.03	3.64	5.35	3.8
Histidine	5.782	8.8	2.5	7.54	2.3	8.5	2.1	4	2.2	1.74	2.5	1.7
Isoleucine	1.037	0.9	2.6	0.64	5	0	2	2.6	4.8	4.56	4.91	0
Leucine	11.63	13.6	7	13.11	7.1	14.9	9.5	12.3	7.84	6.78	8.66	15.6
Phenylalanine	5.749	10.7	4.1	6.28	4.6	9.6	4.7	6.6	4.63	4.64	4.19	3.2
Cysteine			2.5	0.61	1.5	0.9	2.3	5.9	1.52	1.15	1.46	14
Arginine			4.2	4	6.7	3.5	5.8	5.9	5.85	4.35	7.15	7

52-70 w/w% (per whole blood mass) plasma can be produced during the separation of whole blood depending on the technology and the product requirements (Ockerman & Hansen, 1988). Plasma usually does not contain the blood cells so does not contain the hemoglobin from the erythrocytes either. The colour of plasma is not red like fresh whole blood and has no iron taste and blood smell. It is mostly not water-clear due to the residual hemoglobin but it is light-brown or pale pink. If the colorization of final productmade with blood plasma, is not preferred, it can be improved by decolorization of the raw blood plasma. The pH of raw plasma is 6.9, the water content of raw plasma is 91.53 w/w% and the ash content of raw plasma is 0.66 w/w%. The tea-coloured, decolorized plasma has 7.04 pH, 92.64 w/w% water content and 1.53% ash content. The protein content decreases from 5.97 w/w% to 4.61 w/w% during the decolourization process (Makara et al., 2016). High salt content of blood plasma powder (about 15 w/w%) must be considered in when setting plasma powder levels of the recipes, because salt content of egg white is about 0.31 w/w% and salt content of whole egg is about 0.2 w/w%. Egg white has 12.8 w/w%, egg yolk has 16.1 w/w% and whole egg has 9.45 w/w% protein content (Rodler, 2006). 54-55 w/w% of egg white is albumin. Egg yolk proteins contain 14% serum albumin, 41% glycoprotein and 45% immunoglobulin (Jolivet et al., 2008). Comparison of essential amino acid composition of blood and egg fractions and meat myosin are shown in table 1.

The aim of this study is to substitute egg as an allergen by blood plasma in sponge cake. For this purpose sponge cakes were made with egg by traditional method and with egg powder as well as with blood plasma powder by industrial method and these samples were investigated. In this research techno-functional and sensory attributes were measured by instrumental methods. These attributes were the followings: water activity, dry matter content, texture (chewiness, hardness) and colour.

Material and methods

Material

Sample groups of sponge cakes were prepared according to three different recipe, which are shown in table 2.

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

Ingredients	Traditional 'hand made' sponge cake	Sponge cake with egg powder	Sponge cake with whole blood plasma powder
Fine flour (g)	23.7	23.47	23.31
Crystal sugar of normal particle size (g)	18.96	18.78	18.65
Salt (g)	0.47	0.47	0.47
Egg (g)	56.87	-	-
Egg powder (g)	-	13.15	-
Sodium bicarbonate (g)	-	1.88	1.87
Jilk paste (g)	-	0.94	0.93
Water (g)	-	41.31	41.41
Blood plasma powder (g)	-	-	8.39
Sunflower oil (g)	-	-	4.97

Egg powder was provided by Capriovus Kft., Hungary and plasma powder 70B was a obtained from Sonac Burgum B.V., Netherlands.

Methods

Determination of the recipe

The aim was to substitute allergenic egg protein by blood plasma protein. At the same time fat content of egg has been substituted as well with sunflower oil, which has a naturalistic flavour. Water content of egg was replaced by water in case of each recipe. Thus the sponge cake matrix surrounding the proteins was closely the same as with egg. Raw material specifications and food nutrition database (USDA, 2018) were used for calculating the recipe. We calculated with average M sized eggs, which are 58 g of weight.

Sponge cake preparation

In case of 'hand made' cakes egg white and egg yolk were separated. Egg yolk was mixed with the 1/3 part of the sugar and egg white was whipped with 2/3 part of the sugar, then the two parts were softly mixed with the sifted flour. In case of industrial cakes[1], ingredients

[1] 'Industrial cake' means that the method of production is similar to industrial method, but it did not made in large volume but in small quantities in a laboratory.

were mixed well with jilk paste (bakery product emulsifier ingredient), then a foamy mass was made from the mixture by a household mixer. Sponge cake batter was filled into a baking sheet. Sponge cakes were baked at 180 °C for 20 min in a convection oven. Sponge cakes were stored for four days, because fresh bakery products are expected to be stored for maximum four days before consumption. The storage conditions corresponded to the average expected home storage of fresh bakery products: at room temperature in imperfectly sealed (folded) plastic bag. More samples were baked from the same sample groups on consecutive days to eliminate the change in room humidity.

Water activity measurement

Water activity was measured by Novasina LabMaster-aw neo type instrument (Novasina AG, Switzerland) that requires very small sample amount and can fully control the temperature between 0 and 60 °C during the measurement. Measurements were performed at room temperature to control the integrity of samples for relevant data collection.

Moisture content measurement

3-5 g samples, measured by Kern ABJ-NM/ABS-N (Kern & Sohn GmbH, Germany) analytical balance, were put into open Petri dishes. Then the samples were dried at 120 °C until constant mass in a laboratory drying oven (Labor Műszeripari Művek, Hungary). Samples were cooled in a desiccator then their residual mass was measured by the analytical balance. Each sample variety was measured in triplicate.

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 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 numbered making colours analysable.

The instrument was calibrated with a standard white etalon. Each sample was measured three times. Measured attributes are the followings: redness/greenness (a*), yellowness/blueness (b*) and brightness (L*).

Texture measurement

Texture of sponge cakes was measured by Stable Micro System (SMS) TA.XT Plus texture analyser (Stable Micro System, United Kingdom). A computer controlled the movement of the upper rod. Different probes are helping measurements with different shear, compressive and torsional stresses in different directions lasting different times while behaviour of samples was investigated.

Sponge cake samples had upward widening cylindrical shape with 5 cm basis diameter and an average height of 10 cm. The probe was a p/75v steel cylinder plate, which started to press the sample with 0.002 N force. The extent of the load was 40% of sample height. The pre-test speed of the probe was 10 mm/s and test speed and post-test speed 0.5 mm/s. 'Hold until time' type compression measurement method was used with 30 s compressing time. Then the sample relaxed. Sampling frequency was 50/s. Each sample was measured three times.

Crust hardness was calculated from the force, which caused 40% deformation in the sample height, and the extent of this deformation. Cake flexibility was calculated from the ratio of original height and relaxed height of muffins after 30 s 40% deformation.

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 raw material and storage time on redness-greenness, yellowness-blueness, brightness and dry matter content in the first analysis and on crust hardness and cake flexibility in the second analysis, we carried out multivariate analysis of variance (MANOVA), 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

Water activity of samples, which is shown in table 3, was relatively stable. The greatest change occurred in the traditional 'hand made' sponge cake. According to the MANOVA storage time after baking was not a significant factor but the type of the cake was considered as a significant factor. Tukey post hoc test could separate two groups: traditional 'hand made' and cakes with plasma powder were in the first group and cakes with egg powder were in the second group.

Table 3. Measured water activity [-] values of different type samples in case of different storage time [day]

Sample type	Storage time after baking [day]	Mean of water activity [-]	Std. deviation of water activity [-]
Sponge cake with egg powder	0	0.9172	0.0511
	1	0.8969	0.0223
	2	0.9026	0.0153
	3	0.9083	0.0211
Traditional ‘hand made’ sponge cake	0	0.8424	0.0243
	1	0.8839	0.0161
	2	0.9012	0.0057
	3	0.8819	0.0037
Sponge cake with whole blood plasma powder	0	0.8583	0.0206
	1	0.8812	0.0112
	2	0.8833	0.0039
	3	0.8775	0.0166

Moisture content, which is shown in table 4, was relatively stable as well in case of these samples. Storage time was not identified as a significant parameter by MANOVA. Type of sponge cake was a slightly significant parameter. Tukey post hoc test could separate two groups: traditional sponge cake samples and samples made with plasma powder were in the first group and samples with plasma powder and the ones with egg powder were in the second group. Thus, samples with plasma powder were classified into both groups.

Table 4. Measured water activity [w/w%] values of different type samples in case of different storage time [day]

Storage time after baking [day]	Sample type	Mean of moisture content [w/w%]	Std. deviation of moisture content [w/w%]
0	Sponge cake with egg powder	0.324	0.0409
	Traditional ‘hand made’ sponge cake	0.322	0.0358
	Sponge cake with whole blood plasma powder	0.304	0.0306

1	Sponge cake with egg powder	0.341	0.0749
	Traditional ‘hand made’ sponge cake	0.294	0.0352
	Sponge cake with whole blood plasma powder	0.301	0.0412
2	Sponge cake with egg powder	0.341	0.0379
	Traditional ‘hand made’ sponge cake	0.325	0.0339
	Sponge cake with whole blood plasma powder	0.314	0.014
3	Sponge cake with egg powder	0.359	0.0427
	Traditional ‘hand made’ sponge cake	0.283	0.0330
	Sponge cake with whole blood plasma powder	0.319	0.0156

There was not significant difference between sample groups with different sample types and different storage times based on the results of colour measurement. Colour parameters of different sample groups were very similar.

In case of hardness, the results of which are shown in table 5., measured values were hardly evaluable because of the great standard deviation. There were too many overhanging plots to eliminate. Normality of the unstandardized residuals could not be verified. However, the difference between samples with plasma powder and group of other samples is clear and great.

Table 5. Measured hardness [N mm^{-1}] values of different type samples in case of different storage time [day]

Storage time after baking [day]	Sample type	Mean of hardness [N mm^{-1}]	Std. deviation of hardness [N mm^{-1}]
0	Sponge cake with egg powder	5.38	0.002
	Traditional ‘hand made’ sponge cake	3.78	1.429
	Sponge cake with whole blood plasma powder	33.85	4.985

1	Sponge cake with egg powder	13.04	16.741
	Traditional ‘hand made’ sponge cake	4.91	1.183
	Sponge cake with whole blood plasma powder	31.16	0.195
2	Sponge cake with egg powder	5.85	1.782
	Traditional ‘hand made’ sponge cake	3.99	0.773
	Sponge cake with whole blood plasma powder	23.44	5.024
3	Sponge cake with egg powder	4.97	1.442
	Traditional ‘hand made’ sponge cake	4.99	0.808
	Sponge cake with whole blood plasma powder	27.04	6.629

In case of the other texture measurement, a significant parameter was not found either, because conditions for the MANOVA were not met. But if breaking force values were eliminated, a significant parameter was found for compressive stress at the time of breaking, namely the sample type. Samples with plasma powder could be significantly separated from other sample groups based on the compressive stress at the time of breaking. Measured values of braking force and compressive stress at the time of breaking can be found in table 6.

Table 6. Value of measured breaking force [N s] and compressive stress at the time of breaking [mPa] in case of different type samples and different storage time [day]

Parameter	Sample type	Storage time after baking [day]	Mean	Std. deviation
Breaking force [N s]	Sponge cake with egg powder	0	97.68	12.784
		1	107.17	47.461
		2	168.40	31.098
		3	161.82	97.522
	Traditional ‘hand made’ sponge cake	0	139.16	72.028
		1	235.79	44.348
		2	164.59	67.957
		3	278.95	87.9

	Sponge cake with whole blood plasma powder	0	192.19	17.105
		1	148.41	53.869
		2	164.91	59.825
		3	190.06	41.269
Compressive stress at the time of breaking [mPa]	Sponge cake with egg powder	0	5.25	0.113
		1	5.29	1.687
		2	8.45	1.022
		3	7.69	3.374
	Traditional 'hand made' sponge cake	0	7.6	2.968
		1	12.84	3.394
		2	8.83	4.189
		3	13.38	4.435
	Sponge cake with whole blood plasma powder	0	11.1	1.112
		1	11.31	2.853
		2	10.51	2.562
		3	9.92	3.428

Conclusion

Based on this research the allergenic egg powder can be substituted by non-allergenic blood plasma powder in sponge cakes, but the change in the ingredient has an effect on some properties. If instrumentally measured sensory attributes of different types of cakes were compared, cakes made with plasma powder were found harder and more firm, more suitable for instance cake sculpting or producing harder cakes, which can withstand higher load from fillings. Water activity stayed near the critical 0.86 value, which is the lowest water activity value, where human-pathogenic microorganism (coagulase positive *Staphylococcus aureus*) can produce toxin (Deák et al., 2006). Moisture content also remained on a desirable level. Product development was successful. However, this topic needs further investigations: organoleptic quality has to be validated by a sensory analysis according to the GSP, because high salt content of blood plasma powder can cause non-compliances beside inadequate fillings or flavouring. Salted caramel, salted hazelnut and salted chocolate flavouring are especially adequate for the natural cake with plasma powder.

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