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Application of arginine and histidine to improve the technological and sensory properties of low-fat and low-sodium bologna-type sausages produced with high levels of KCl

Suelem Lima da Silva[a], José Manuel Lorenzo[b], Juliana Missio Machado[a], Marialene Manfio[a], Alexandre José Cichoski[a], Leadir Lucy Martins Fries[a], Marcelo Antonio Morgano[c], Paulo Cezar Bastianello Campagnol[a,*]

[a] Universidade Federal de Santa Maria, CEP 97105-900 Santa Maria, Rio Grande do Sul, Brazil
[b] Centro Tecnológico de la Carne de Galicia, Parque Tecnológico de Galicia, San Cibrán das Viñas, Rúa Galicia N 4, Ourense, Spain
[c] Instituto de Tecnologia de Alimentos, CEP 13070-178, Campinas, São Paulo, Brazil

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ABSTRACT

Low-fat bologna sausages were made with the replacement of 60% NaCl by KCl and with the addition of arginine and histidine alone or in combination. The technological and sensory properties were evaluated. The replacement of NaCl by KCl did not significantly affect the color parameters L*, a*, and b*. However, the emulsion stability, the texture profile, and the sensory quality were impaired by the salt substitution. The addition of arginine and histidine alone or in combination was effective to reduce the defects caused by the addition of KCl. The present study demonstrated that bologna sausages with reduced sodium content (≈40%) and acceptable technological and sensory properties can be produced using 1% NaCl, 1.5% KCl, 1% arginine and 0.2% histidine.

1. Introduction

Excess consumption of foods containing high levels of saturated fat and sodium is considered a precursor to chronic diseases (Bowen, Sullivan, Kris-Etherton, & Petersen, 2018). Thus, public health regulatory agencies of several countries have recommended the food industry reduce the use of these ingredients in their products. In Latin America, Chile was the pioneer in establishing that processed foods with high content of sugar, saturated fat and sodium must include on the front of their package separate black orthogonal signs with the expression "High in…" for each nutrient. In addition, the country has banned the inclusion of these foods in school meals, as well as advertising in mass media publication and programs for children under 14 years of age (de Salud, 2015). This law has affected the meat industry, and led to major debates and political and economic pressures, from both an national and international point of view. For these reasons, reducing the saturated fat and sodium content of meat products remains an urgent need for the meat industry.

Sodium chloride (NaCl) is the main source of sodium in emulsified meat products, thus the reduction of this ingredient contributes to healthier products. However, this is not an easy task, NaCl reduces water activity and so it has a great impact on shelf life and confers the salty taste characteristic of meat products. In addition, it assists in the extraction of myofibrillar proteins, thus contributing to achieve the desired technological and sensory properties (Tobin, O'Sullivan, Hamill, & Kerry, 2012). The search for a substitute that maintains the same properties of NaCl is a great challenge for the meat industry. A number of approaches have been adopted, and one of the most effective is the partial replacement of NaCl with potassium chloride (KCl) (Lorenzo, Cittadini, Bermúdez, Munekata, & Domínguez, 2015; Yu, Xu, Jiang, & Xia, 2017). The great advantage of this approach is that potassium consumption is associated with a reduction in blood pressure (Whelton et al., 1997), however, KCl should be consumed with caution by consumers with renal disease due of hyperkalemia risks (Bianchi et al., 2019). Another advantage is that KCl has antimicrobial properties similar to NaCl (Bidlas & Lambert, 2008) and does not affect the shelf life and product safety (Lorenzo, Cittadini, et al., 2015). However, the main disadvantage of KCl is decrease the sensory quality of meat products by reducing the salty taste and conferring a bitter, astringent, and metallic taste at NaCl replacement levels above 50% (Santos Alves et al., 2017).

The use of amino acids has been shown to be an interesting alternative to reduce the sensory defects caused by high levels of NaCl

* Corresponding author.
E-mail address: paulo.campagnol@ufsm.br (P.C.B. Campagnol).

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replacement by KCl. Lysine stands out among the amino acids most used for this purpose, which was effective to reduce the sensory defects caused by the substitution of 50% NaCl by KCl in fermented (Campagnol, Wagner, Terra, & Pollonio, 2011; Dos Santos, Campagnol, Morgano, & Pollonio, 2014) and emulsified (Santos Alves et al., 2017) meat products. Arginine and histidine are also amino acids with great potential to be applied as flavor enhancers in meat products. However, the combined application of arginine and histidine in meat products has been little explored in the literature. Arginine was effective in masking the sensory defects caused by KCl in cheese (Felicio et al., 2016) and was useful to improve emulsion stability and TPA parameters of emulsified sausages (Zhu et al., 2018). On the other hand, histidine was able to improve the water distribution and the volatile compounds of meat products (Liu et al., 2019; Zhu et al., 2018). In this context, the combination of these amino acids may be effective for producing meat products with reduced sodium content. This approach can also bring other nutritional benefits, arginine can prevent cardiovascular and renal diseases (Pamarin, 2006) and histidine may prevent obesity (Li et al., 2016), reduce inflammation (Niu et al., 2012) and blood pressure (Tuttle, Milton, Packard, Shuler, & Short, 2012) and improve insulin resistance and reduced oxidative stress (Feng et al., 2013).

In a previous study, we used a fat substitute made from pork skin, water, and high oleic sunflower oil in bologna sausages. It was possible to reduce about 29% of fat, 10% of cholesterol, and 21% of the energy value of the product, without compromising its technological and sensory quality. In addition, a healthier lipid profile was reached (Lima da Silva et al., 2019). However, to give even healthier characteristics to this product, it is necessary to reduce its sodium content. Although the application of amino acids on meat products is not a good economic alternative, studies reported that consumers are willing to pay a high price for sausages reduced in their salt and fat content (Schnettler et al., 2019). Thus, this study produced low-fat bologna-type sausages with 60% NaCl replacement by KCl. In addition, the amino acids arginine and histidine were used alone or in combination. The effect of this reformulation on the technological and sensory properties of bologna sausages was evaluated.

2. Materials and methods

2.1. Manufacture of bologna sausages

Low-fat bologna sausages were prepared according to the formulation and procedures described by Lima da Silva et al. (2019). The control bologna sausage ($Control_{NaCl}$) was elaborated with 2.5% NaCl. The modified treatments were made with the replacement of 60% NaCl by KCl and with the addition of 1% arginine and/or 0.2% histidine (Table 1). These replacement level of NaCl by KCl was chosen to ensure that the $Control_{KCl}$ had a lower technological and sensory quality than the $Control_{NaCl}$. The choice of dose rates for arginine and histidine were defined in preliminary studies. Ten sausages (200 ± 20 g) of each treatment per replicate (n = 3) were produced.

2.2. Proximate composition and pH

The moisture (AOAC Method 950.46), fat (AOAC Method 991.36), protein (AOAC Method 2011.04), and ash (AOAC Method 920.153) contents of the bologna sausages were determined in triplicate (Association of Official Analytical Chemists, 2006a, 2006b, 2006c, 2006d). The pH was determined in triplicate using 5 g of sample, which was homogenized with 50 mL of distilled water, and the pH was measured using a digital pH meter (DM-23-DC, Digimed, Brazil).

2.3. Determination of sodium and potassium

The quantification of sodium and potassium was performed in triplicate using an inductively coupled plasma emission spectrometer (ICP-OES) (Agilent, model 5100 VDV ICP OES, Agilent Technologies, Tokyo, Japan). The operating conditions were: power of 1200 W; nebulization rate of 0.7 L min^{-1}; primary and auxiliary argon flux of 12 and 1 L min^{-1}; stabilization time and reading time of 15 and 7 s. The wavelengths (nm) used were: Na (589,592) and K (766,491) (Association of Official Analytical Chemists, 2005).

Table 1
Formulation of low-fat Bologna-type sausages produced with replacement of 60% NaCl by KCl and addition of arginine and histidine.

(%)	$Control_{NaCl}$	$Control_{KCl}$	Arg	His	Arg + His
Pork meat	65	65	65	65	65
Pork back fat	10	10	10	10	10
Fat replacer[a]	10	10	10	10	10
NaCl	2.5	1	1	1	1
KCl	0	1.5	1.5	1.5	1.5
Arginine	0	0	1	0	1
Histidine	0	0	0	0.2	0.2
Sodium tripolyphosphate	0.3	0.3	0.3	0.3	0.3
Sodium nitrite	0.015	0.015	0.015	0.015	0.015
Sodium erythorbate	0.025	0.025	0.025	0.025	0.025
Coriander	0.2	0.2	0.2	0.2	0.2
Black pepper	0.1	0.1	0.1	0.1	0.1
Ice	11.86	11.86	10.86	11.66	10.66
Total	100	100	100	100	100

[a] Pork skin: water: High oleic sunflower oil (1.5:1.5:1). Chemical composition: Pork meat (moisture: 71.31% ± 0.1; protein: 20.74% ± 0.12; fat: 3.01 ± 0.09); Pork back fat (moisture: 10.87% ± 0.1; protein: 8.12% ± 0.07; fat: 90.12 ± 0.41); Fat replacer (moisture: 50.68% ± 0.1; protein: 25.21% ± 0.42; fat: 18.12% ± 0.22).

2.4. Emulsion stability

The emulsion stability was determined in triplicate using the following equations: (%) Fat release: (weight of released fat/weight of sample) × 100; (%) Water release: (weight of water released / weight of sample) × 100 (Jiménez-Colmenero, Ayo, & Carballo, 2005).

2.5. Texture profile analysis

The samples were cut into cylinders 2 cm high × 2 cm wide. The texture profile analysis was performed in nine cylinders of each treatment using the TA-TX2 texture analyzer (Stable Micro Systems Ltd., Surrey, England). The cylinders were compressed twice at 1 mm/s to 50% of their original height with a P/45 probe (45 mm Ø). The results were obtained through a force x time curve, and the parameters hardness (N / cm^2), elasticity (cm), cohesiveness, and chewing (N/cm) were calculated.

2.6. Instrumental color

The values of L* (lightness), a* (redness) and b* (yellowness) were determined using a colorimeter (CM-700D, A illuminant and 10° observation angle, Konica-Minolta Ltd., Osaka, Japan) with round aperture size of 1.5 cm diameter. Color values were measured in the internal portion of the bologna sausages (nine readings at different points of each treatment).

2.7. Sensory evaluation

One hundred habitual consumers of meat products (55 female and 45 male, aged from 18 to 65 years) participated of the sensory evaluation. The tests were performed in individual booths with fluorescent lighting (350 lx). Samples (0.5 cm thickness) coded with three random numbers were served to consumers in a sequential monadic form, following a Latin square design (Macfie, Bratchell, Greenhoff, & Vallis, 1989). Water and crackers were also provided to consumers for palate cleansing.

Consumers first performed the acceptance test using a structured 9-point hedonic scale (1 = extremely disliked and 9 = extremely liked). Samples were evaluated for the attributes color, aroma, flavor, texture, and overall acceptance (Stone, Bleibaum, & Thomas, 2012). Consumers completed a check-all-that-apply (CATA) questionnaire containing the following descriptors: pale color, ideal color, pleasant aroma, unpleasant aroma, rancid flavor, bitter taste, pleasant taste, unpleasant taste, metallic taste, astringent taste, a little salt, salt in the right measure, ideal texture, and succulent. These terms were previously generated by a group of seven habitual consumers of bologna sausage.

2.8. Statistical analysis

The experiment was repeated three times on three different days ($n = 3$). Data were analyzed through analysis of variance (Anova) using a general linear model. For the technological properties, the treatments were considered as a fixed effect and the replicates as a random effect. For the consumer test, treatments were considered as a fixed effect and consumers as a random variable. No significant difference was found between consumers. The Tukey's test was used at the 5% level of significance when significant differences were found. To evaluate the CATA results a contingency table was obtained with the frequency of each term for each sample. Then, a correspondence analysis was performed using the chi-square distance (Vidal, Tarrega, Antunez, Ares, & Jaeger, 2015).

3. Results and discussion

The results of the proximate composition (Table 2) showed that the NaCl replacement by KCl and the addition of arginine and/or histidine did not affect the moisture and fat contents when compared to the traditional bologna sausage ($Control_{NaCl}$) ($P > .05$). The fat content of bologna sausages was close to 12% (Table 2). It is worth mentioning that the fat content of the bologna sausages of this study was about 60% lower than the maximum allowed by Brazilian legislation (Brasil, 2000). Although the treatments Arg and Arg + His had a higher protein content ($P < .05$) when compared to the other treatments, this may be overestimated as these amino acids have about 32% (arginine) and 27% (histidine) nitrogen in their constitution. The treatments with the addition of amino acids also presented a slightly higher ash content ($P < .05$) than the control treatments (ControlNaCl and ControlKCl).

The present results also demonstrated that one of the main objectives of the study was reached, the amount of sodium of the treatments made with NaCl replacement by KCl reduced by up 47.1% (Table 2). Thus, all treatments with NaCl replacement by KCl can be labeled with the claim "reduced sodium content" (European Parliament, 2006). In addition, the partial replacement of NaCl with KCl increased potassium levels by > 200%, which is also beneficial for the nutritional quality of the product because an increased potassium intake may mitigate the negative consequences of high sodium consumption (WHO, 2012).

In emulsified meat products, the emulsion stability and the texture profile are related to the ability to retain water and fat after the heat treatment (Horita, Morgano, Celeghini, & Pollonio, 2011), and the ionic strength is a key factor influencing these properties (Hamm, 1986). The concentrations of NaCl normally added to meat products produce the ionic strength required for the dissolution and extraction of the myofibrillar proteins responsible for emulsification, gelatinization, and water retention capacity (Feiner, 2006). In this study, fat exudation was not affected ($P > .05$) by the NaCl replacement by KCl (Table 2). However, an increase in water exudation was observed in the $Control_{KCl}$ treatment. This result demonstrates that the substitution of 60% of NaCl by KCl should be carried out with caution because it can reduce the emulsion stability. The higher water exudation led to changes in the texture profile, the $Control_{KCl}$ presented higher ($P < .05$) hardness values when compared to the $Control_{NaCl}$. These findings can be due to the lower ionic strength of $Control_{KCl}$ (0.37) in relation to $Control_{NaCl}$ (0.43) (Horita et al., 2011).

The treatments with the addition of arginine and histidine alone presented emulsion stability and texture profile ($P > .05$) similar to the $Control_{NaCl}$. In addition, the combined addition of arginine and histidine (Arg + His) presented lower ($P < .01$) water exudation and hardness when compared to $Control_{NaCl}$. The addition of arginine and histidine increased ($P < .001$) the pH values (Table 2) and deviated from isoelectric point of myofibrillar proteins (Puolanne & Halonen, 2010), which may justify the improvement in these technological properties. Moreover, the lower water exudation can be explained by the formation of hydrogen bond and ion-dipole interactions between

Table 2
Effect of the replacement of NaCl by KCl and addition of amino acids (arginine and histidine) on proximate composition, sodium, potassium, emulsion stability, texture profile and color of low-fat Bologna-type sausages.

	$Control_{NaCl}$	$Control_{KCl}$	Arg	His	Arg + His	SEM	*P*-value
Moisture (%)	64.9^{a}	64.6^{a}	64.0^{a}	64.1^{a}	63.2^{a}	0.1	n.s.
Fat (%)	12.1^{a}	12.1^{a}	12.6^{a}	11.8^{a}	12.3^{a}	0.2	n.s.
Protein (%)	18.6^{c}	18.4^{c}	20.1^{b}	18.5^{c}	20.8^{a}	0.2	***
Ash (%)	3.7^{b}	3.7^{b}	3.8^{a}	3.8^{a}	3.8^{a}	0.02	*
Sodium (mg/100 g)	1164.2^{a}	657.4^{b}	616.2^{b}	704.1^{b}	646.1^{b}	12.1	***
Potassium (mg/100 g)	293.1^{b}	977.5^{a}	994.4^{a}	950.1^{a}	1089.2^{a}	4.3	***
Emulsion stability							
Fat exudation (%)	0.79^{a}	0.78^{a}	0.79^{a}	0.80^{a}	0.79^{a}	0.01	n.s.
Water exudation (%)	3.8^{b}	4.7^{a}	3.9^{b}	3.9^{b}	3.1^{c}	0.06	**
pH	5.73^{c}	5.75^{c}	5.85^{ab}	5.79^{b}	5.91^{a}	0.01	***
Hardness (N/cm^2)	92.5^{b}	94.2^{a}	92.5^{b}	91.8^{b}	90.6^{c}	0.5	*
Springiness (cm)	0.57^{a}	0.6^{a}	0.54^{a}	0.57^{a}	0.61^{a}	0.04	n.s
Cohesiveness (dimensionless)	0.77^{a}	0.78^{a}	0.79^{a}	0.78^{a}	0.79^{a}	0.2	n.s.
Chewiness (N/cm)	61.3^{bc}	62.3^{ab}	61.6^{abc}	60.7^{c}	62.9^{a}	0.5	**
L*	60.1^{a}	60.9^{a}	59.3^{a}	60.8^{a}	60.6^{a}	0.8	n.s.
a*	20.1^{a}	19.8^{a}	21.2^{a}	19.6^{a}	19.7^{a}	0.7	n.s.
b*	15.7^{a}	15.6^{a}	15.7^{a}	15.4^{a}	15.2^{a}	0.4	n.s.

Averages within the same line followed by the same letters did not show any significant difference ($P > .05$) by Tukey's test. Batches: $Control_{NaCl}$: 2.5% NaCl; $Control_{NaCl}$: 1% NaCl, and 1.5% KCl; Arg: 1% NaCl, 1.5% KCl, and 1% arginine; His: 1% NaCl, 1.5% KCl, and 0.2% histidine; Arg: 1% NaCl, 1.5% KCl, 1% arginine, and 0.2% histidine.
SEM-standard error of the mean.
P-value: *** ($P < .001$), ** ($P < .01$), * ($P < .05$), n.s. (not significant).

Table 3
Results of consumer study (n = 100) of low-fat Bologna-type sausages produced with replacement of 60% NaCl by KCl and addition of arginine and histidine.

	$Control_{NaCl}$	$Control_{KCl}$	Arg	His	Arg + His	SEM	P-value
Color	7.0[a]	6.8[a]	6.7[a]	6.7[a]	6.7[a]	< 0.1	n.s.
Aroma	6.6[a]	5.5[b]	6.2[ab]	6.2[ab]	6.2[ab]	< 0.1	*
Flavor	6.6[a]	5.6[b]	6.2[ab]	6.5[a]	6.4[ab]	< 0.1	***
Texture	6.2[a]	5.5[b]	5.8[ab]	6.3[a]	5.9[a]	< 0.1	**
Overall acceptability	6.6[a]	5.6[b]	6.5[a]	6.4[a]	6.1[ab]	< 0.1	***

Averages within the same line followed by the same letters did not show any significant difference (P > .05) by Tukey's test. Batches: $Control_{NaCl}$: 2.5% NaCl; $Control_{NaCl}$: 1% NaCl, and 1.5% KCl; Arg: 1% NaCl, 1.5% KCl, and 1% arginine; His: 1% NaCl, 1.5% KCl, and 0.2% histidine; Arg: 1% NaCl, 1.5% KCl, 1% arginine, and 0.2% histidine.

the side chain of these amino acids and water (Araújo, 2012). Furthermore, it is likely that the amino acids increased water retention due to their ability to increase the extraction of myofibrillar proteins (Guo, Peng, Zhang, Liu, & Cui, 2015).

The L*, a*, and b* values were not affected by the substitution of NaCl by KCl and the addition of arginine and histidine (Table 2). These results are very positive as consumers tend to reject products outside the traditional coloring patterns. In agreement with these results, Santos Alves et al. (2017) and Horita et al. (2011) also found no differences in L*, a* and b* values of emulsified meat products when replacing 50% NaCl by KCl.

KCl is the main NaCl substitute in meat products. However, it is well documented that levels from 50% replacement may decrease the sensory quality of meat products (Gelabert, Gou, Guerrero, & Arnau, 2003; Guardia, Guerrero, Gelabert, Gou, & Arnau, 2008). In this study, as expected, the treatment with 60% NaCl replacement by KCl ($Control_{KCl}$) had significantly lower sensory scores when compared to the $Control_{NaCl}$ for the attributes aroma, flavor, texture, and overall acceptance (Table 3). On the other hand, no significant differences were observed in the sensory evaluation of the treatments with the addition of arginine and histidine alone or in combination, when compared to the $Control_{NaCl}$. In agreement with these results, Zhou, Li, and Tan (2014) reported a positive influence of the addition of arginine on the sensory characteristics of sausages. In addition, Feiner (2006) also reported that arginine was effective in minimizing the metallic taste resulting from the addition of large amounts of KCl in low-sodium cheese. Fig. 1 shows the treatments and the descriptors of the first and second dimensions of the correspondence analysis (CA) performed to evaluate the CATA results. The CA explained 95.78% of the total variance, which was explained by the first dimension (F1: 91.69%). The treatments were separated into F1 in two distinct groups. The group located in the negative quadrant of the first dimension (F1) was formed by the $Control_{NaCl}$ and the treatments containing arginine and histidine (Arg, His and Arg + His). The $Control_{KCl}$ was located in the positive quadrant of F1.

The $Control_{KCl}$ was characterized by low salt, metallic flavor, astringent taste, unpleasant aroma, and rancid aroma. These descriptors are commonly reported in studies using KCl as a salt substitute (Ruusunen & Puolanne, 2005; Santos Alves et al., 2017) and therefore may explain the lower sensory acceptance of this treatment (Table 3). The treatments $Control_{NaCl}$, Arg, His and Arg + His were characterized by the descriptors salt in the right measure, pleasant taste, succulent, and ideal texture. These descriptors may explain the high sensory acceptance of these treatments (Table 3) and demonstrate that the addition of arginine and histidine masked the consumers' perception of the major sensory defects related to the use of KCl as a salt substitute.

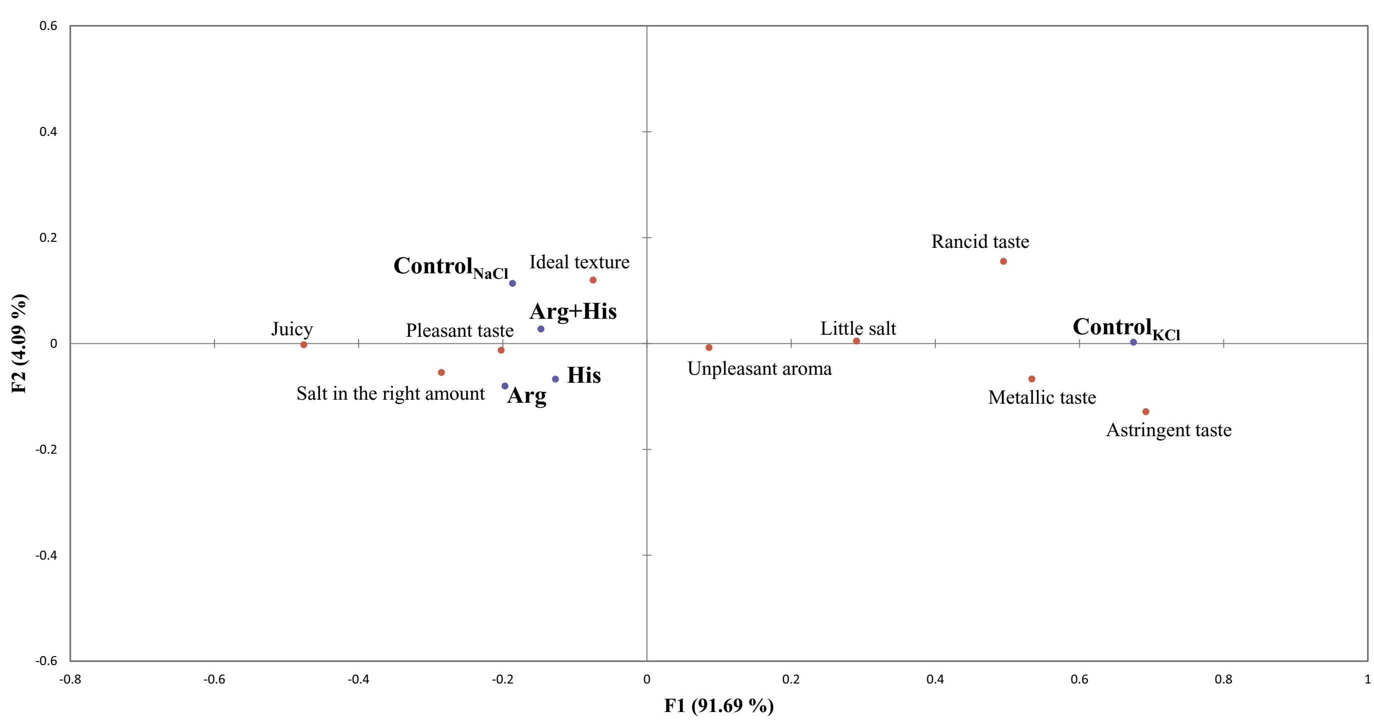

Fig. 1. Correspondence analysis on CATA questions of the low-fat Bologna-type sausages produced with replacement of 60% NaCl by KCl and addition of arginine and histidine.

4. Conclusion

The combined effect of the addition of arginine and histidine on the technological and sensory quality of emulsified meat products with NaCl replacement by KCl was evaluated in this study. The results demonstrated that the replacement of 60% NaCl by KCl impaired the emulsion stability, the texture profile, and the sensory quality of the processed products. The addition of arginine and histidine alone or in combination was effective to reduce the defects caused by the addition of KCl. Therefore, the reformulation proposed in this study can be considered a viable alternative for the elaboration of emulsified meat products with good technological and sensory properties and healthier characteristics. However, further studies are needed to evaluate the shelf life of the reformulated sausages and the effect of arginine and histidine on the formation of biogenic amines.

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Declarations of Competing Interest

None.

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