

# An exploratory study of pre-extrusion flavouring: investigation with vitamins, amino acids, essential oils, natural aromas and seasonings

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**ABSTRACT.** Expanded corn snacks are obtained through thermoplastic extrusion and then a lipid vehicle (oil or hydrogenated vegetable fat) is sprinkled on the product, giving the desired flavour. However, this second step called post-extrusion flavouring increases the lipid content and the caloric value of the snack, reasons why these products are highly criticized. Thus, the study of pre-extrusion flavourings is an interesting way of finding corn snacks with better nutritive value. Therefore, we aimed to explore pre-extrusion flavouring using different substances, investigating the odour and flavour on the extrudates and the impact of such substances on their expansion ratio, density, and colour. To do this, vitamins, amino acids, essential oils, natural aromas and seasonings were added to corn grits and extruded. The addition of different substances, especially the essential oils, natural aromas and seasonings, produced odour and flavour in the extrudates, as well as having little negative impact on their physical properties of the extrudates, no impact or even a small positive impact. Therefore, this study provides a range of possibilities to be explored, using pre-extrusion flavouring as a way of reducing the use of lipids and improving the nutritive value of expanded snacks.

**Keywords:** aroma precursor; expansion ratio; density; colour.

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## Introduction

Thermoplastic extrusion is a versatile technique with high production, low costs and is an environmentally safe process. The basic principle of such technology consists of the conversion of a solid material to a fluid one, applying heat and mechanical work during conduction of the material along a barrel to a die. In consequence, starch gelatinization, protein denaturation and reorientation, enzyme inactivation, destruction of antinutritional factors and microbial count decreasing occur. In the end, the sudden fall from the internal pressure to atmospheric pressure leads to water evaporation, which, together with the die configuration, cut speed and extrusion conditions, results in a product with the desired characteristics (Fellows, 2000).

As a result of this high versatility and flexibility, thermoplastic extrusion has been used in the production of many varied products in the food industry, with expanded corn snacks standing out. After extrusion, the extrudates are flavoured using a commercial aroma dissolved in a lipid vehicle (oil or hydrogenated vegetable fat), that it is sprinkled on the product, providing the desired flavour (Bhandari, D'Arcy, & Young, 2001; Brennan, Derbyshire, Tiwari, & Brennan, 2013). Although the flavour of snacks is one of the most important factors in the popularity of these products (Delcour & Hoskeney, 2010; Menis-Henrique, Janzanti, & Conti-Silva, 2017), this kind of flavouring method (post-extrusion) increases the lipid content and the caloric value of the snack, reasons why these products are highly criticized (Moubarac et al., 2012). Therefore, pre-extrusion flavouring methods could be interesting for producing healthier extruded snacks because the use of lipids for flavouring can be minimized or eliminated.

Pre-extrusion flavouring can be performed by adding different types of flavouring agents to the raw material to be extruded: commercial aromas or volatile compounds (Yuliani, Torley, & Bhandari, 2009; Conti-Silva, Bastos, & Areas, 2012; Menis, Milani, Jordano, Boscolo, & Conti-Silva, 2013; Milani, Menis, Jordano, Boscolo, & Conti-Silva, 2014), encapsulated volatile compounds (Yuliani, Bhandari, Rutgers, & D'Arcy, 2004; Yuliani, Torley, D'Arcy, Nicholson, & Bhandari, 2006a; Yuliani, Torley, D'Arcy, Nicholson, &

Bhandari, 2006b), and aroma precursors (Bredie, Hassell, Guy, & Mottram, 1997; Farouk, Pudil, Janda, & Pokorný, 2000; Majcher, Ławrowski, & Jeleń, 2010; Davidek, Festrings, Dufossé, Novotny, & Blank, 2013; Menis-Henrique et al., 2019). Pre-extrusion flavouring enables a uniform distribution of volatile compounds in the product, as well as improving the stability against oxidation, since they are encapsulated within the matrix of the extruded product. On the other hand, loss of volatile compounds may occur during the extrusion process, requiring the addition of higher amounts of flavouring agents, increasing costs, and also adversely affecting the extrudate structure and texture (Bhandari et al., 2001), reasons that are considered disadvantages of pre-extrusion flavouring.

Our research group has been studying pre-extrusion flavouring for a while, and had already obtained interesting results (Conti-Silva et al., 2012; Menis et al., 2013; Milani et al., 2014; Menis-Henrique et al., 2019). Moreover, other results about feasibility of adding aroma precursors to corn grits and soy protein concentrate are at the stage to be published. In this way, the investigation of different agents for flavouring to snacks using the pre-extrusion flavouring technique must be widened, in order to expand the knowledge in the area of thermoplastic extrusion. Therefore, we aimed to explore pre-extrusion flavouring using different substances: vitamins, amino acids, essential oils, natural aromas and seasonings; investigating the odour and flavour in the extrudates, as well the impact of such substances on their physical properties, knowing that the pre-extrusion flavouring may affect the structure and texture of extrudates. In the end, we aimed becoming pre-extrusion flavouring a feasible technique for producing snacks with lower lipid content and better nutritive value than those produced using post-extrusion flavouring.

## Material and methods

### Material

The corn grits (Master SP Alimentos, Capela do Alto, Brazil) were obtained from the local market in São José do Rio Preto (Brazil). The vitamins (ascorbic acid, biotin, folic acid, niacin, riboflavin, phyloquinone, pyridoxine, thiamine, and tocopherol) and the amino acids (arginine, cysteine, glutamine, glycine, isoleucine, leucine, lysine, methionine, Mg aspartate, phenylalanine, proline, tryptophan, tyrosine, and valine), all powder and pure, were purchased from a compounding pharmacy in São José do Rio Preto (Brazil). The essential oils (bay leaf, coriander, cumin, garlic, ginger, natural smoke, nutmeg, oregano, rosemary, and sweet paprika; all in liquid form), the natural aromas (bacon, basil, ginger, lemon, onion, and smoked; all in powder form), and the seasonings (cumin, curry, ginger, powder bay leaf, rosemary, saffron, and spicy paprika; powder or dehydrated) were obtained through donations made by the Duas Rodas (Jaraguá do Sul, Brazil) and Doremus (Guarulhos, Brazil) companies.

Vitamins and amino acids were tested as aroma precursors. Essential oils, natural aromas and seasonings were tested in order to verify the use of such already flavoured substances for production of the extrudates. In this study, the vitamins, amino acids, essential oils, natural aromas, and seasonings are, collectively, called substances, for facilitating understanding.

### Preparation of corn grits for extrusion processing

Firstly, the corn grits had the moisture adjusted to 15% on a dry basis. All the powdered substances studied (vitamins, amino acids, natural aromas and seasonings) were added to corn grits in the proportion of 1.2% (w w<sup>-1</sup>). The liquid substances (essential oils) were mixed with water at a proportion of 1.67% (v v<sup>-1</sup>), as recommended by the company Doremus, in order to solubilize themselves in water. After that, the essential oils were added to corn grits in the same proportion of 1.2% (v w<sup>-1</sup>). The proportion of substances added, similar to that used by Conti-Silva et al. (2012), Menis et al. (2013), Milani et al. (2014), who used 1.5% of each volatile compound, was defined at 1.2% in function of preliminary tests in order to reduce the intense bitter taste of some substances. After addition of the substances, the corn grits were maintained at room temperature for 2 h before extrusion.

### Extrusion processing

The corn grits were extruded on different days for each group of substances (vitamins, amino acids, essential oils, natural aromas, and seasonings), using pure corn grits for each sample to guarantee the non-

interference of the previously extruded mixtures. Moreover, the extrusion of corn grits with each substance was randomized at each day for extrusion.

The corn grits were extruded in an RX PQ Labor 24 single screw extruder with semi-industrial capacity (Inbramaq – *Indústria de Máquinas Ltda*, Ribeirão Preto, Brazil). The conditions were similar to those used by Menis-Henrique et al. (2019): helicoidally grooved barrel; screw with a large step of one exit with a compression ratio of 2.3:1 and length-to-diameter ratio of 15.5:1; pre-die extruder with holes of 3.01 mm; extruder die with a diameter of 2.93 mm (round hole); feed rate of 265 g min.<sup>-1</sup>; screw speed at 192 rpm; temperatures from zone 1 to zone 5: 50, 70, 90, 120, and 120°C, respectively. A control extrudate was obtained through the exclusive extrusion of corn grits (without any added substance).

### Analysis of odour and flavour in the extrudates

Considering that this is an exploratory study about pre-extrusion flavouring, the extrudates were submitted to an informal sensory analysis, just to evaluate the production of odour and flavour after extrusion. Odour is the perception of the volatile compounds through orthonasal olfaction and of the chemical sensing by the trigeminal nerve when a food is approximated to the nostrils and the air inspired. On the other hand, the flavour involves the combination of gustative perception of soluble and non-volatile compounds (basic tastes), volatile compounds perceived through retronasal olfaction (aroma) and chemical sensations through the trigeminal nerve. Therefore, five members of the research group smelled and tasted the extrudates, in order to identify if any odour and flavour was produced during extrusion.

### Analysis of the physical properties of the extrudates

The expansion ratio was determined for ten replicates, being the ratio between the diameter of the extrudate and the diameter of the extruder die. The density was determined in ten extrudates, using the equation  $\rho = 4M/\pi D^2L$ , in which M = extrudate mass (g), D = extrudate diameter (cm), and L = extrudate length (cm). An IP54 digital caliper (Digimess, São Paulo, Brazil) was used to obtain the expansion ratio and density.

The extrudates were submitted to colour analysis, using a Color Flex colorimeter (HunterLab, Reston, USA), with illuminant D65 and a 10° observer angle. The CIE-L\*a\*b\* system (L\* = luminosity, C\* = chrome, and h = hue) was used. The extrudates were milled, placed in a circular quartz capsule with an internal diameter of 58 mm, and analyzed in quadruplicate. Each replicate was rotated four times (0°, 90°, 180°, and 270°), resulting in sixteen values for each sample.

### Statistical analysis

The means of the physical properties were compared between the extrudates obtained through pre-extrusion flavouring with each substance and the control extrudate. For that, the means were submitted to the Student t test for independent samples, assuming different variations at a significance level 0.05, using the Microsoft Office Excel 2016 software.

## Results and discussion

### Vitamins

The addition of ascorbic acid, folic acid and riboflavin resulted in extrudates with odour/flavour of lemon, lemon, and intense bitter taste respectively (Table 1). On the other hand, the others vitamins did not produce any odour/flavour besides the extruded corn flavour. Both ascorbic acid and folic acid produced odour/flavour of lemon, which may be related to the chemical function of carboxylic acid in the substances, resulting in volatile compounds found in lemon. The ascorbic acid is described as providing constant sour taste, tingling, sharp, acidic, and fruity (Schiffman & Dackis, 1975). For riboflavin, oxi-reduction cyclic reactions may have resulted in quinone derivatives (Damodaran, Parkin, & Fennema, 2007), bitter substances that lead to the bitter taste in the extrudate. Moreover, riboflavin is described as a bitter substance, involving attributes described as dry, mineral and stale (Schiffman & Dackis, 1975).

**Table 1.** Sensory characteristics and physical properties (mean  $\pm$  SD) of the extrudates obtained through pre-extrusion flavouring with vitamins.

	Odour/flavour	Expansion ratio <sup>a</sup>	Density (g cm <sup>-3</sup> ) <sup>a</sup>	Colour <sup>a</sup>		
				L*	C*	hour (°)
Control	Extruded corn	4.1 <sup>a</sup> $\pm$ 0.2	0.201 <sup>a</sup> $\pm$ 0.016	81.8 <sup>a</sup> $\pm$ 0.3	42.5 <sup>a</sup> $\pm$ 0.3	83.3 <sup>a</sup> $\pm$ 0.2
Ascorbic acid	Lemon	4.0 <sup>a</sup> $\pm$ 0.3	0.170 <sup>a</sup> $\pm$ 0.028	81.1 <sup>b</sup> $\pm$ 0.2	36.3 <sup>b</sup> $\pm$ 0.2	78.5 <sup>b</sup> $\pm$ 0.1
Biotin	-	4.3 <sup>a</sup> $\pm$ 0.4	0.092 <sup>b</sup> $\pm$ 0.017	82.4 <sup>b</sup> $\pm$ 0.5	35.4 <sup>b</sup> $\pm$ 0.7	78.5 <sup>b</sup> $\pm$ 0.3
Folic acid	Lemon	4.0 <sup>a</sup> $\pm$ 0.5	0.083 <sup>b</sup> $\pm$ 0.008	78.7 <sup>b</sup> $\pm$ 0.6	48.5 <sup>b</sup> $\pm$ 0.4	82.9 <sup>b</sup> $\pm$ 0.6
Niacin	-	3.9 <sup>a</sup> $\pm$ 0.4	0.130 <sup>b</sup> $\pm$ 0.034	85.3 <sup>b</sup> $\pm$ 0.2	30.6 <sup>b</sup> $\pm$ 0.1	81.6 <sup>b</sup> $\pm$ 0.1
Riboflavin	Intense bitter taste	3.9 <sup>a</sup> $\pm$ 0.6	0.197 <sup>a</sup> $\pm$ 0.040	75.5 <sup>b</sup> $\pm$ 0.1	88.8 <sup>b</sup> $\pm$ 0.4	76.6 <sup>b</sup> $\pm$ 0.1
Phylloquinone	-	4.2 <sup>a</sup> $\pm$ 0.4	0.149 <sup>b</sup> $\pm$ 0.027	80.6 <sup>b</sup> $\pm$ 0.3	44.2 <sup>b</sup> $\pm$ 0.5	85.3 <sup>b</sup> $\pm$ 0.1
Pyridoxine	-	2.6 <sup>b</sup> $\pm$ 0.3	0.093 <sup>b</sup> $\pm$ 0.021	88.3 <sup>b</sup> $\pm$ 0.1	21.2 <sup>b</sup> $\pm$ 0.2	84.5 <sup>b</sup> $\pm$ 0.1
Thiamine	-	3.9 <sup>a</sup> $\pm$ 0.2	0.158 <sup>b</sup> $\pm$ 0.015	84.6 <sup>b</sup> $\pm$ 0.1	34.7 <sup>b</sup> $\pm$ 0.3	83.9 <sup>b</sup> $\pm$ 0.1
Tocopherol	-	4.2 <sup>a</sup> $\pm$ 0.4	0.142 <sup>b</sup> $\pm$ 0.025	83.9 <sup>b</sup> $\pm$ 0.2	43.2 <sup>b</sup> $\pm$ 0.3	84.0 <sup>b</sup> $\pm$ 0.1

<sup>a</sup>Expansion ratio and density (n = 10); colour (n = 16). The symbol (-) indicates that the extrudate showed no different odour/flavour from the control. Different letters in the same column indicate significantly different means between the experimental extrudate and the control by the Student t test (p  $\leq$  0.05).

The expansion ratio and the density are used for evaluating the degree of expansion of the extrudates because the expansion ratio considers the expansion on the cross section of the extrudate while the density considers it in all directions. Thus, less dense extrudates tend to be more expanded and have a more brittle structure or less mechanical resistance, which are desirable characteristics for expanded snacks (Yuliani et al., 2009; Meng, Threinen, Hansen, & Driedger, 2010). Regarding the expansion ratio, the addition of vitamins did not result in significant changes in the extrudates in relation to the control, except the extrudate with pyridoxine (Table 1), whose expansion ratio decreased. However, for most of the extrudates, the density significantly decreased in relation to the control, even the extrudate obtained with pyridoxine that had a lower expansion ratio. Different functional groups are present in the vitamins studied, such as alcohol, amine, amide, carboxylic acid, aldehyde and ketone. Moreover, more than one group is common per molecule. However, pyridoxine only has three hydroxyl groups and this may be the reason for the reduction in the expansion ratio in relation to the control. Kim and Maga (1994), cited by Bhandari et al. (2001), studying the retention of a series of volatile compounds, with varying chain lengths and functional groups, in a high amylose starch during extrusion, found that interactions between alcohol and aldehyde groups and starch influenced the expansion of the extrudate.

The densities of all extrudates were significantly lower in relation to the control, probably due to the functional groups of the vitamins interacting with the starch during the extrusion process. At the end of extrusion, the main interaction between starch molecules, and it is considered as responsible for the structure of the final product, is hydrogen bond between glucose residues, interaction that is relatively weak and that result in highly expanded products and with fragile structure (Fellows, 2000). All the vitamins (except phylloquinone) have hydroxyl groups in the molecule, which may have favoured the occurrence of hydrogen bonds with starch during extrusion. Considering that the expansion ratio did not change (except for pyridoxine) and the density was reduced, this interaction probably made the internal layer of the extrudate become thinner, incorporating more air in the vacuoles and, thus, reducing the density of the extrudates. Therefore, the extrudate structure was not affected by the addition of vitamins before extrusion, as described by Bhandari et al. (2001).

The extrudates obtained through extrusion with the vitamins had different colours from the control (Table 1), with a significant increase or decrease in the values of luminosity (L\*), chroma (C\*) and hue (h). The extrudate with riboflavin stood out regarding chroma, which had a high value of 88.8. Indeed, the riboflavin is an orange powder and it can be used as a food dye (Aberoumand, 2011). However, even with significant differences in the colour attributes of the extrudates, all the hue angles ranged from 70° to 100°, indicating that all extrudates could be characterized as having a yellow colour (Ramos & Gomide, 2017) that is characteristic of corn extrudates.

### Amino acids

Many of the amino acids resulted in odour/flavour in the extrudates (Table 2), some intriguing, such as liver from cysteine, although meaty aromas had been found in extrudates obtained through extrusion of wheat flour with cysteine and reducing sugars added (Bredie et al., 1997) or in roasted products (Hofmann & Schierberle, 1998). The odour/flavour of some cruciferous vegetables, like kale, cauliflower and cabbage, as well as cress (The Good Scents Company - <http://www.thegoodscentscompany.com>, accessed November 18,

2019) results from volatile compounds with sulphur in the molecule. Thus, knowing that methionine is a sulphur amino acid, the odour/flavour of cauliflower must result from that. However, the mechanisms for kale, cabbage and cress odours were not defined because isoleucine, Mg aspartate and phenylalanine are not sulphurous molecules. There are already snacks with vegetable flavours available in Brazil because of tendencies towards vegetarianism/veganism and more natural products. Thus, our results are promising, especially considering that the flavouring method for these commercial snacks is still post-extrusion, using a lipid vehicle. Lysine and tryptophan had a bitter taste and, indeed, free amino acids may confer a bitter taste as well as sweetness (Yang et al., 2019). Odours described as caramel, spicy, sulphuric, sharp, pungent and other adjectives, were found in extrudates obtained through the extrusion of wheat flour, glucose, and different amino acids (Farouk et al., 2000). In relation to chlorine (from arginine) and slightly piquant (from leucine), no information about such odour/ flavor was found at Flavornet (<http://www.flavornet.org/flavornet.html>, accessed November 18, 2019) or at The Good Scents Company for suggesting possible mechanisms of formation of the volatile compounds.

Most of the extrudates had significant differences for the expansion ratio in relation to the control (Table 2), both higher and lower. However such effects were not strictly aligned with the type of the amino acid. Amino acids may be classified according to their polarity (Damodaran et al., 2007). Nonpolar amino acids (glycine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, and valine) reduced or maintained the expansion ratio in relation to the control. Among the polar amino acids, arginine (polar, with positive charge) and glutamine (polar, no charge) enhanced the expansion ratio, contrary to the other polar amino acids (aspartate, cysteine, lysine, and tyrosine), which reduced it. In spite of the lower expansion ratio of some extrudates with other amino acids, the results here are relevant since the maximum expansion ratios shown by starchy extrudates obtained through pre-extrusion flavouring were 2.2 (Yuliani et al., 2009), 3.7 (Conti-silva et al., 2012), and 3.1 (Menis et al., 2013). However, the extrudate with lysine must be noted as it had low expansion (2.0) considering that a starchy raw material was extruded. This result is not desired, especially considering that cereals, such as corn, have lysine as a limiting essential amino acid. Thus, the use of lysine as a fortifier is not feasible considering the negative impact on its expansion ratio of the extrudate.

Although there were differences in the expansion ratio, the density of all the extrudates was significantly lower in relation to the control. As with vitamins, all amino acids have a carboxyl functional group and the hydroxyl group may have interacted with the starch during the extrusion processing, making the internal layer of the extrudate thinner, incorporating more air in the vacuoles and, thus, reducing the density of the extrudates.

Changes in the colour attributes were observed for extrudates obtained with the addition of amino acids in relation to the control (Table 2). However, all the hue angles ranged from 70° to 100°, indicating that all extrudates could be characterized as having a yellow colour that is characteristic of corn extrudates.

**Table 2.** Sensory characteristics and physical properties (mean  $\pm$  SD) of the extrudates obtained through pre-extrusion flavouring with amino acids.

	Odour/ flavour	Expansion ratio <sup>a</sup>	Density (g cm <sup>-3</sup> ) <sup>a</sup>	Colour <sup>a</sup>		
				L*	C*	hour (°)
Control	Extruded corn	4.1 <sup>a</sup> $\pm$ 0.2	0.201 <sup>a</sup> $\pm$ 0.016	81.8 <sup>a</sup> $\pm$ 0.3	42.5 <sup>a</sup> $\pm$ 0.3	83.3 <sup>a</sup> $\pm$ 0.2
Arginine	Chlorine	4.6 <sup>b</sup> $\pm$ 0.5	0.137 <sup>b</sup> $\pm$ 0.031	82.8 <sup>b</sup> $\pm$ 0.5	32.8 <sup>b</sup> $\pm$ 0.5	84.6 <sup>b</sup> $\pm$ 0.5
Cysteine	Liver	3.7 <sup>b</sup> $\pm$ 0.3	0.167 <sup>b</sup> $\pm$ 0.028	81.4 <sup>b</sup> $\pm$ 0.1	26.6 <sup>b</sup> $\pm$ 0.5	84.5 <sup>b</sup> $\pm$ 0.1
Glutamine	-	4.5 <sup>b</sup> $\pm$ 0.3	0.163 <sup>b</sup> $\pm$ 0.036	81.3 <sup>a</sup> $\pm$ 0.7	40.7 <sup>b</sup> $\pm$ 2.1	83.1 <sup>b</sup> $\pm$ 0.3
Glycine	-	4.3 <sup>a</sup> $\pm$ 0.2	0.176 <sup>b</sup> $\pm$ 0.020	81.9 <sup>a</sup> $\pm$ 1.3	37.9 <sup>b</sup> $\pm$ 1.2	84.0 <sup>b</sup> $\pm$ 0.8
Isoleucine	Kale <sup>1</sup>	4.1 <sup>a</sup> $\pm$ 0.3	0.121 <sup>b</sup> $\pm$ 0.019	80.9 <sup>b</sup> $\pm$ 0.3	32.1 <sup>b</sup> $\pm$ 0.3	83.6 <sup>b</sup> $\pm$ 0.2
Leucine	Slightly piquant	3.9 <sup>b</sup> $\pm$ 0.1	0.129 <sup>b</sup> $\pm$ 0.008	84.2 <sup>b</sup> $\pm$ 0.8	41.3 <sup>b</sup> $\pm$ 2.0	84.0 <sup>b</sup> $\pm$ 0.3
Lysine	Bitter taste	2.0 <sup>b</sup> $\pm$ 0.3	0.087 <sup>b</sup> $\pm$ 0.023	80.7 <sup>b</sup> $\pm$ 0.7	35.8 <sup>b</sup> $\pm$ 1.0	83.5 <sup>b</sup> $\pm$ 0.3
Methionine	Cauliflower <sup>2</sup>	3.9 <sup>b</sup> $\pm$ 0.2	0.170 <sup>b</sup> $\pm$ 0.020	83.9 <sup>b</sup> $\pm$ 0.2	42.9 <sup>b</sup> $\pm$ 0.4	83.2 <sup>b</sup> $\pm$ 0.1
Mg aspartate	Cabbage <sup>3</sup>	3.7 <sup>b</sup> $\pm$ 0.3	0.117 <sup>b</sup> $\pm$ 0.040	75.7 <sup>b</sup> $\pm$ 0.2	27.7 <sup>b</sup> $\pm$ 0.3	77.8 <sup>b</sup> $\pm$ 0.1
Phenylalanine	Cress	3.6 <sup>b</sup> $\pm$ 0.2	0.141 <sup>b</sup> $\pm$ 0.019	82.9 <sup>b</sup> $\pm$ 0.3	39.6 <sup>b</sup> $\pm$ 0.5	84.0 <sup>b</sup> $\pm$ 0.1
Proline	-	4.3 <sup>a</sup> $\pm$ 0.3	0.163 <sup>b</sup> $\pm$ 0.031	81.7 <sup>a</sup> $\pm$ 1.2	40.2 <sup>b</sup> $\pm$ 2.0	83.5 <sup>a</sup> $\pm$ 0.7
Tryptophan	Sweet and bitter taste	4.3 <sup>a</sup> $\pm$ 0.4	0.143 <sup>b</sup> $\pm$ 0.024	82.3 <sup>b</sup> $\pm$ 0.3	41.6 <sup>b</sup> $\pm$ 0.7	82.9 <sup>b</sup> $\pm$ 0.2
Tyrosine	-	3.4 <sup>b</sup> $\pm$ 0.3	0.114 <sup>b</sup> $\pm$ 0.019	83.9 <sup>b</sup> $\pm$ 0.4	35.0 <sup>b</sup> $\pm$ 0.2	84.2 <sup>b</sup> $\pm$ 0.2
Valine	-	3.8 <sup>b</sup> $\pm$ 0.3	0.154 <sup>b</sup> $\pm$ 0.017	82.6 <sup>b</sup> $\pm$ 0.7	40.5 <sup>b</sup> $\pm$ 0.4	83.1 <sup>b</sup> $\pm$ 0.4

<sup>a</sup>Expansion ratio and density (n = 10); colour (n = 16). The symbol (-) indicates that the extrudate showed no different odour/flavour from the control. <sup>1</sup>*Brassica oleracea* var. *acephala*; <sup>2</sup>*Brassica oleracea* var. *botrytis* L.; <sup>3</sup>*Brassica oleracea* var. *capitata*. Different letters in the same column indicate significantly different means between the experimental extrudate and the control by the Student t test (p  $\leq$  0.05).

### Essential oils, natural aromas, and seasonings

Almost all the essential oils (Table 3) and the natural aromas (Table 4), and all the seasonings (Table 5) resulted in odour/flavour similar to that of the substance itself, except the essential oils coriander, ginger, and natural smoke, which did not have an odour different from extruded corn, and the natural aroma of basil, which presented the odour/flavour of fennel. Volatile compounds related to the odours of coriander, ginger and smoke are linalyl formate, 3 - (benzyl oxy) - 2,2 - dimethyl propanal and 2,3-dimethyl benzofuran respectively (Flavornet; The Good Scents Company). The boiling point of linalyl formate is 100°C (Lide & Frederikse, 1997), indicating that losses may have occurred during extrusion. However, the boiling point of 2,3-dimethyl benzofuran is around 200°C (Lide & Frederikse, 1997), and of 3-(benzyl oxy)-2,2-dimethyl propanal is 283.2°C (ChemSpider - <http://www.chemspider.com/Chemical-Structure.9980228.html>, accessed November 24, 2019), so it is not possible to infer about losses of such compounds during extrusion. Even so, one alternative may be to study the use of higher concentrations of such substances. Chemical reactions must be happening, when changing the odour/flavour from basil to fennel.

**Table 3.** Sensory characteristics and physical properties (mean  $\pm$  SD) of the extrudates obtained through pre-extrusion flavouring with essential oils.

	Odour/flavour	Expansion ratio <sup>a</sup>	Density (g cm <sup>-3</sup> ) <sup>a</sup>	Colour <sup>a</sup>		
				L*	C*	hour (°)
Control	Extruded corn	4.1 <sup>a</sup> $\pm$ 0.2	0.201 <sup>a</sup> $\pm$ 0.016	81.8 <sup>a</sup> $\pm$ 0.3	42.5 <sup>a</sup> $\pm$ 0.3	83.3 <sup>a</sup> $\pm$ 0.2
Bay leaf	Bay leaf	4.5 <sup>b</sup> $\pm$ 0.3	0.130 <sup>b</sup> $\pm$ 0.016	83.8 <sup>b</sup> $\pm$ 0.2	41.2 <sup>b</sup> $\pm$ 0.4	83.9 <sup>b</sup> $\pm$ 0.2
Coriander	-	3.5 <sup>b</sup> $\pm$ 0.3	0.239 <sup>b</sup> $\pm$ 0.045	83.0 <sup>b</sup> $\pm$ 0.5	40.6 <sup>b</sup> $\pm$ 0.1	83.3 <sup>b</sup> $\pm$ 0.4
Cumin	Cumin	5.0 <sup>b</sup> $\pm$ 0.2	0.116 <sup>b</sup> $\pm$ 0.013	83.5 <sup>b</sup> $\pm$ 0.3	40.4 <sup>b</sup> $\pm$ 0.6	84.3 <sup>b</sup> $\pm$ 0.2
Garlic	Garlic	5.1 <sup>b</sup> $\pm$ 0.5	0.115 <sup>b</sup> $\pm$ 0.017	82.2 <sup>b</sup> $\pm$ 0.4	40.1 <sup>b</sup> $\pm$ 0.0	84.8 <sup>b</sup> $\pm$ 0.2
Ginger	-	4.4 <sup>b</sup> $\pm$ 0.3	0.137 <sup>b</sup> $\pm$ 0.016	82.6 <sup>b</sup> $\pm$ 0.6	41.2 <sup>b</sup> $\pm$ 1.4	83.4 <sup>b</sup> $\pm$ 0.4
Natural smoke	-	4.1 <sup>a</sup> $\pm$ 0.3	0.160 <sup>b</sup> $\pm$ 0.017	83.5 <sup>b</sup> $\pm$ 0.3	39.5 <sup>b</sup> $\pm$ 0.1	83.9 <sup>b</sup> $\pm$ 0.3
Nutmeg	Nutmeg	3.9 <sup>b</sup> $\pm$ 0.3	0.165 <sup>b</sup> $\pm$ 0.035	83.8 <sup>b</sup> $\pm$ 0.2	40.2 <sup>b</sup> $\pm$ 0.6	84.0 <sup>b</sup> $\pm$ 0.2
Oregano	Oregano	4.8 <sup>b</sup> $\pm$ 0.2	0.116 <sup>b</sup> $\pm$ 0.011	83.4 <sup>b</sup> $\pm$ 0.7	40.8 <sup>b</sup> $\pm$ 1.0	84.1 <sup>b</sup> $\pm$ 0.5
Rosemary	Rosemary	4.4 <sup>b</sup> $\pm$ 0.1	0.128 <sup>b</sup> $\pm$ 0.009	83.1 <sup>b</sup> $\pm$ 0.4	40.8 <sup>b</sup> $\pm$ 0.6	83.8 <sup>b</sup> $\pm$ 0.2
Sweet paprika	Sweet paprika	4.2 <sup>b</sup> $\pm$ 0.3	0.132 <sup>b</sup> $\pm$ 0.014	80.0 <sup>b</sup> $\pm$ 0.4	38.1 <sup>b</sup> $\pm$ 3.4	74.6 <sup>b</sup> $\pm$ 0.7

<sup>a</sup>Expansion ratio and density (n = 10); colour (n = 16). The symbol (-) indicates that the extrudate showed no different odour/flavour from the control. Different letters in the same column indicate significantly different means between the experimental extrudate and the control by the Student t test (p  $\leq$  0.05).

**Table 4.** Sensory characteristics and physical properties (mean  $\pm$  SD) of the extrudates obtained through pre-extrusion flavouring with natural aromas.

	Odour/flavour	Expansion ratio <sup>a</sup>	Density (g cm <sup>-3</sup> ) <sup>a</sup>	Colour <sup>a</sup>		
				L*	C*	hour (°)
Control	Extruded corn	4.1 <sup>a</sup> $\pm$ 0.2	0.201 <sup>a</sup> $\pm$ 0.016	81.8 <sup>a</sup> $\pm$ 0.3	42.5 <sup>a</sup> $\pm$ 0.3	83.3 <sup>a</sup> $\pm$ 0.2
Bacon	Bacon	4.5 <sup>b</sup> $\pm$ 0.2	0.174 <sup>b</sup> $\pm$ 0.018	81.2 <sup>b</sup> $\pm$ 0.4	41.7 <sup>b</sup> $\pm$ 0.7	82.3 <sup>b</sup> $\pm$ 0.3
Basil	Fennel	4.3 <sup>a</sup> $\pm$ 0.2	0.195 <sup>a</sup> $\pm$ 0.025	82.3 <sup>b</sup> $\pm$ 0.3	43.2 <sup>b</sup> $\pm$ 0.8	82.8 <sup>b</sup> $\pm$ 0.2
Ginger	Ginger	4.1 <sup>a</sup> $\pm$ 0.4	0.210 <sup>a</sup> $\pm$ 0.033	81.0 <sup>b</sup> $\pm$ 0.3	43.2 <sup>b</sup> $\pm$ 0.8	82.3 <sup>b</sup> $\pm$ 0.2
Lemon	Lemon	4.4 <sup>b</sup> $\pm$ 0.3	0.175 <sup>b</sup> $\pm$ 0.023	79.5 <sup>b</sup> $\pm$ 1.0	40.4 <sup>b</sup> $\pm$ 1.2	83.0 <sup>b</sup> $\pm$ 0.6
Onion	Onion	4.6 <sup>b</sup> $\pm$ 0.5	0.137 <sup>b</sup> $\pm$ 0.031	82.3 <sup>b</sup> $\pm$ 0.2	42.8 <sup>b</sup> $\pm$ 0.4	82.7 <sup>b</sup> $\pm$ 0.1
Smoked	Smoked	3.6 <sup>b</sup> $\pm$ 0.4	0.308 <sup>b</sup> $\pm$ 0.060	79.9 <sup>b</sup> $\pm$ 0.3	42.5 <sup>a</sup> $\pm$ 1.0	82.1 <sup>b</sup> $\pm$ 0.2

<sup>a</sup>Expansion ratio and density (n = 10); colour (n = 16). The symbol (-) indicates that the extrudate showed no different odour/flavour from the control. Different letters in the same column indicate significantly different means between the experimental extrudate and the control by the Student t test (p  $\leq$  0.05).

**Table 5.** Sensory characteristics and physical properties (mean  $\pm$  SD) of the extrudates obtained through pre-extrusion flavouring with seasonings.

	Odour/flavour	Expansion ratio <sup>a</sup>	Density (g cm <sup>-3</sup> ) <sup>a</sup>	Colour <sup>a</sup>		
				L*	C*	hour (°)
Control	Extruded corn	4.1 <sup>a</sup> $\pm$ 0.2	0.201 <sup>a</sup> $\pm$ 0.016	81.8 <sup>a</sup> $\pm$ 0.3	42.5 <sup>a</sup> $\pm$ 0.3	83.3 <sup>a</sup> $\pm$ 0.2
Cumin	Cumin	4.1 <sup>a</sup> $\pm$ 0.1	0.160 <sup>b</sup> $\pm$ 0.012	79.8 <sup>b</sup> $\pm$ 0.6	37.2 <sup>b</sup> $\pm$ 0.8	83.3 <sup>b</sup> $\pm$ 0.4
Curry	Curry	4.2 <sup>a</sup> $\pm$ 0.3	0.190 <sup>a</sup> $\pm$ 0.029	78.8 <sup>b</sup> $\pm$ 1.3	41.3 <sup>b</sup> $\pm$ 1.2	84.7 <sup>b</sup> $\pm$ 0.9
Ginger	Ginger	4.2 <sup>a</sup> $\pm$ 0.3	0.177 <sup>b</sup> $\pm$ 0.025	78.9 <sup>b</sup> $\pm$ 0.8	39.2 <sup>b</sup> $\pm$ 3.3	83.9 <sup>b</sup> $\pm$ 0.5
Powder bay leaf	Powder bay leaf	4.2 <sup>a</sup> $\pm$ 0.3	0.201 <sup>a</sup> $\pm$ 0.019	76.8 <sup>b</sup> $\pm$ 1.8	38.4 <sup>b</sup> $\pm$ 1.9	83.7 <sup>b</sup> $\pm$ 1.0
Rosemary	Rosemary	4.2 <sup>a</sup> $\pm$ 0.4	0.177 <sup>b</sup> $\pm$ 0.031	78.1 <sup>b</sup> $\pm$ 0.5	40.6 <sup>b</sup> $\pm$ 1.0	83.7 <sup>b</sup> $\pm$ 0.2
Saffron	Saffron	4.1 <sup>a</sup> $\pm$ 0.3	0.220 <sup>a</sup> $\pm$ 0.035	80.6 <sup>b</sup> $\pm$ 0.7	59.8 <sup>b</sup> $\pm$ 0.3	86.2 <sup>b</sup> $\pm$ 0.4
Spicy paprika	Spicy paprika	4.2 <sup>a</sup> $\pm$ 0.3	0.172 <sup>b</sup> $\pm$ 0.024	76.8 <sup>b</sup> $\pm$ 0.4	37.4 <sup>b</sup> $\pm$ 0.3	78.7 <sup>b</sup> $\pm$ 0.3

<sup>a</sup>Expansion ratio and density (n = 10); colour (n = 16). The symbol (-) indicates that the extrudate showed no different odour/flavour from the control. Different letters in the same column indicate significantly different means between the experimental extrudate and the control by the Student t test (p  $\leq$  0.05).

The maintenance of the odour/flavour of essential oils, aroma natural, and seasonings after extrusion is interesting, because it means it is possible to produce extrudates that have market appeal, such as bacon, onion and smoked, which are currently flavoured using a lipid vehicle. However, it is important to mention that all the flavours perceived should be considered as 'fugacious', resulting in a momentary perception of volatile compounds inside the mouth which is explained by the absence of lipids in the extrudates. The release of lipophilic volatile compounds is slower when the amount of lipid in the product is higher, resulting in a long-lasting perception of volatile compounds and, consequently, of the flavour (Menis-Henrique et al., 2019). Therefore, studies need to be performed using pre-extrusion flavouring but investigating the role of lipids on the acceptance of the products.

In relation to physical properties, almost all the essential oils and natural aromas promoted a significantly higher expansion ratio and lower density in relation to the control, except for coriander and nutmeg (Table 3), and smoked natural aroma (Table 4). Regarding seasonings (Table 5), all extrudates had expansion ratios not significantly different from the control, as well as lower or similar density in relation to the control. Moreover, all extrudates had similar colour attributes in relation to the control, with hue angles indicating a yellow colour (from 70° to 100°). The effects of essential oils, natural aromas and seasonings were diverse and not specific to each category of substance. For example, among the essential oils, there was a reduction in the expansion ratio with the addition of coriander and nutmeg, no change with the addition of natural smoked aroma and an increase with the addition of all the remaining essential oils. Regarding the density of extrudates with natural aromas, the addition of the smoked aroma increased the density, while the density with basil and ginger stayed the same, and bacon, lemon, and onion reduced the density. Therefore, the effect was specific to the aroma and, considering this study is exploratory, further studies are still necessary for understanding the mechanisms for such specific effects.

## Conclusion

The addition of vitamins, amino acids, essential oils, natural aromas and seasonings before extrusion produced odour and flavour in the extrudates, as well as having little negative impact on the physical properties of the extrudates and, in some cases, no impact or even a small positive impact. Vitamins produced extrudates with odour/flavour of lemon and an intense bitter taste, while amino acids produced extrudates with odour/flavour of chlorine, liver, cruciferous vegetables and piquant, as well as sweet and bitter taste. Essential oils, natural aromas and seasonings maintained their respective original odour/flavour, with the exception of the essential oils coriander, ginger and natural smoke, which lose theirs, and the natural aroma basil, which was perceived as fennel after extrusion. Thus, flavoured extrudates were produced, especially through the addition of essential oils, natural aromas and seasonings, which may find a commercial application, although more studies are needed in order to investigate the sensory acceptance of the snacks. Moreover, the effect of the substances was diverse and not specific to each category of product (i.e., vitamins, amino acids, essential oils, natural aromas, and seasonings). Thus, considering the exploratory nature of this study, further studies could investigate the mechanisms that result in the substance being used as a flavouring ingredient. Finally, this study has provided a range of possibilities for using pre-extrusion flavouring as a way of reducing the use of lipids and improving the nutritive value of snacks.

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