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Vegetable-based frankfurter sausage production by different emulsion gels and assessment of physical-chemical, microbiological and nutritional properties

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A R T I C L E I N F O

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A B S T R A C T

The present work aimed to evaluate emulsion gels from plant sources and their application in frankfurter-type sausages by determining the nutritional, physical-chemical, and microbiological properties of the formulations. Emulsifying capacity and emulsion stability were evaluated with canola oil, water, and three different vegetable-sourced flours (oat, flaxseed, and coconut) to select the emulsion gels. From these results, vegan sausages were formulated to resemble frankfurters and meat Frankfurter-type sausages were employed in the control. Emulsion gels from flaxseed and oat flours displayed high emulsifying capacities and more resistance once the gelling agent was added. Both vegetable-sourced sausages displayed significant differences in protein, fat and sodium. Stability, pH, and water activity did not present significant alterations in 120 days of storage. It was possible to conclude that vegetable-sourced emulsion gels allow the formulation of plant-based sausages with enhanced nutritional properties when compared to traditional meat sausages.

1. Introduction

Veganism has been developing not only in Portugal but also abroad by replacing meat-free meals with meat-type food products. This is easily noticed by the wider offer of these types of products, such as vegan cheese and sausages (Martinho, 2016).

Vegetable-based options are becoming popular because customers are more prone to reduce or avoid the consumption of animal-based food in their diets due to environmental, ethical, or health reasons (Boukid, 2021; Baune et al., 2022). The growing concerns about the risks of developing diseases associated with the consumption of fat-rich food products (mainly saturated fat) have led the industry to advance in creating new products and adding value to the traditional ones (Jiménez-Colmenero et al., 2015).

Traditional frankfurters are commonly made out of red meat which consumption is associated with health risks. Vegan sausages may become possible choices when correctly manufactured to achieve texture and flavor similar to meat sausages and contribute to reducing meat consumption as a promising and ecological option.

To overcome the large consumption of animal fat and encourage people to make healthier choices, many strategies have been proposed in order to reduce animal-derived fat content and improve the lipid profile of products. Among the alternatives are polyunsaturated-fatty-acid-rich oils such as vegetable and extra-virgin olive oils, vegetable-protein-rich fibers such as soya flour, hydrocolloids, and more recently, Emulsion Gels (EGs) (Camara et al., 2020).

Vegetable-sourced gels from soya and wheat protein are an alternative to partially replace the fat content in meat sausages. EGs are emulsions with gel-type structure and solid-type mechanical properties and their preparation consist essentially of an emulsion stabilized by protein with emulsifying agents and the addition of a thickener, such as a hydrocolloid or other gelling ingredients able to create an EG either by aggregating emulsion droplets or continuous-phase gelation (Dickinson, 2013; Jiménez-Colmenero et al., 2015). Gelling agents such as alginate, for example, have proven to be useful in EGs due to their ability to create gel structures at cold temperatures (Pintado et al., 2015, 2016).

Plant-based EGs are employed as a solid substitute for animal fat in vegan sausages. However, the protein and fat chosen to formulate the products play a key role in attaining acceptable texture (Baune et al., 2021). Pintado et al. (2018) and Baune et al. (2019) investigated the function of gels formulated with a range of vegetable flours (chia seeds, oat, peas, and soya), oils (canola and olive oil), and alginate-based stabi-

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lizers and concluded they were successful in partially replacing animal fat in meat sausages. Besides, the final products also exhibited more consistency and less elasticity which corroborates the application of EGs as an alternative to developing meat-type food products.

In this work, the use of emulsion gel from different vegetable sources such as oat, coconut, and flaxseed flours with gelling agents and canola/rapeseed oil is proposed in manufacturing new vegetable-based sausages with added nutritional value.

Thus, the research finds its purpose in the notable search for healthy vegetable-based food products. Since sausages are fat-rich meat products, the development of new alternatives made from plant sources of protein and fat is to be encouraged as well as the assessment of its nutritional, physical-chemical, and microbiological quality analyses.

2. Materials and methods

All raw products were purchased from local markets in Recife (Brazil). Flours (from coconut, oat, and flaxseed) and condiments (garlic, onion, nutmeg, pepper, paprika) and potato were acquired in specialized markets for organic products. Canola oil was purchased from Vitaliv®. The samples were produced and analyzed for physical-chemical and microbiological parameters in the Laboratory of Animal-Based Food Product Technology from the Chemical Engineering Faculty of Universidade Federal de Pernambuco (Brazil).

2.1. Emulsifying activity of vegetable-based flours

Emulsion assays were carried out for three types of vegetable flours (oat, coconut, and flaxseed) according to modifications from Santana et al. (2017) methods. The emulsifying activity was estimated by mechanical vortex agitation (Fisatom model 772). In 15-mL graduated test tubes, 1.0 g of samples were added along with 10 mL of water and 10 mL of canola oil and stirred for 10 min. The supernatant was separated by centrifugation at 3000 rpm for 5 min. The emulsifying activity was calculated according to Eq. 1.

$$Emulsifying\ activity = \frac{emulsion\ layer\ (mL)}{total\ volume\ (mL)}\ x\ 100 \quad (1)$$

2.2. Emulsion stability assays

Emulsion stability was investigated according to Camara et al. (2020) with modifications. Approximately 20 g of emulsion gel was centrifuged for 2 min at 3600 rpm. Then, the samples were heated to 70°C in a water bath for 30 min and centrifuged again for 3 min at 3600 rpm. Emulsion stability was calculated according to Eq. 2.

$$Emulsion\ stability = \frac{Emulsion\ layer\ (mL)}{Total\ volume\ (mL)}\ x\ 100 \quad (2)$$

2.3. Production of emulsion gels

Pintado et al. (2015), 2016) methods were employed to prepare gels by the addition of a gelling agent with different oil/water level. The cold gelling agent was previously prepared by mixing sodium alginate (0.73%)(w/w), calcium sulfate (0.73%) (w/w), and sodium pyrophosphate (0.54%) (w/w) (the last two previously dissolved in water). Formulations are described in Table 1 according to the three vegetable flours: Oat Flour Emulsion Gel (OFEG), Coconut Flour Emulsion Gel (CFEG), and Flaxseed Flour Emulsion Gel (FFEG). Flour level varied between 5% (w/w) and 20% (w/w).

Each sample was prepared in a vortex mixer at 3500 rpm for approximately 1 min. The gelling agent was added for 30 s at 3500 rpm. Canola oil was gradually added at last and then mixed for 1 min. The samples were stored in polyethylene flasks at 5°C.

2.4. Vegetable-based frankfurter production

Two vegetable-sourced frankfurter formulations and one control meat frankfurter were prepared in agreement with NP 723 (2006). Formulae are described in Table 2.

2.5. pH measurements

The determination of pH was carried out by electrometric method in a digital potentiometer (model Bel Engineering PHS3BW). The electrode was brought into contact with crushed samples and the analyses were performed in triplicate.

2.6. Water activity determination

Analyses were carried out directly in a water activity meter (model NOVASINA LabTouch – a_w) in triplicate.

2.7. Color measurements

The color of the samples was evaluated by CIE Lab (International Commission d' Eclairage) system by adding the samples to a color meter (model Tecnal SHE-TEC60CP). The results were given as L* (mean luminosity), a* (redness), and b* (yellowness). Analyses were carried out in triplicate.

2.8. Texture assessment

The texture profile of samples was tested by compression in a texture analyzer (model Brookfield CT3 Texture Analyzer). The probe diameter was 5 mm and the conditions of analyses were at 3.3 $mm.s^{-1}$ at room temperature. Probes were set into 2.5-cm height samples subjected to maximum compression and pressure in N. Analyses were performed in triplicate.

2.9. Determination of humidity and ash content

The humidity levels of samples were estimated by gravimetric analysis. 3.0 g of samples were dried at 105°C as described by AOAC (2000) and NP 1614 (2002). Each sample was analyzed in triplicate. Humidity content (H%) was calculated by Eq. 3 and expressed in g per 100 g.

$$H\% = \frac{P2 - P3}{P2 - P1} x\ 100 \quad (3)$$

Where P_1 represents the weight of the crucible, P_2 is the sum of the crucible and raw sample weights, and P_3 is the crucible and the dried sample mass in grams.

Ash content was evaluated by drying 1.0 g of samples at 550°C in a muffle furnace as described by NP 1615 (2002). Analyses were carried out in triplicate and the ash content was determined in percentages according to Eq. 4.

$$Ash\% = \frac{P1 - P2}{P} x\ 100 \quad (4)$$

Where P_1 represents the weight of the crucible with ash, P_2 is the weight of the crucible itself, and P is the weight of the sample for analyses, all in grams.

2.10. Total lipid determination

The lipid content of samples was determined by the Bligh-Dyer method of cold extraction following NP 1613 (1979). Initially, 2.0 g of crushed samples were dissolved in 10 mL of trichloromethane, 20 mL of methanol, and 8 mL of distilled water by stirring them for 30 min. Then, 10 mL of trichloromethane and 10 mL of sodium sulfate (1.5%) solutions were added and the samples were stirred for 5 min. The extraction of the lipid phase was performed in a decanting funnel and the bottom

Table 1
Emulsion gel formulae.

Sample[a]	Ingredients (%)					
	Oat flour	Coconut flour	Flaxseed flour	Canola oil	Water	Gelling agent
OFEG 1	20	-	-	20	58	2
OFEG 2	5	-	-	52	41	2
CFEG 1	-	20	-	20	58	2
CFEG 2	-	5	-	52	41	2
FFEG 1	-	-	20	20	58	2
FFEG 2	-	-	5	52	41	2

[a] OFEG (Oat Flour Emulsion Gel); CFEG (Coconut Flour Emulsion Gel); FFEG (Flaxseed Flour Emulsion Gel).

Table 2
Control meat sausage (Sample 1) and vegan frankfurter sausages (Samples 2 and 3) formulations. Absent is indicated by a dash.

Ingredients	Rates (%)		
	Sample 1	Sample 2	Sample 3
Mechanically Separated Beef	22.00	-	-
Pork Loin	26.00	-	-
Beef shoulder	16.00	-	-
EG 1	-	38.00	-
EG 2	-	-	38.00
Smashed Potatoes	-	28.05	28.05
Bacon	16.25	-	-
Curing Salt	0.25	0.25	0.25
Ice from Drinkable Water	10.00	10.00	10.00
Cassava Starch	2.00	2.00	2.00
Soya Contrate Protein	0.80	15.00	15.00
Sodium Tripolyphosphate	0.25	0.25	0.25
Sodium erythorbate	0.25	0.25	0.25
Sodium chloride	0.50	0.50	0.50
Onion powder	1.50	1.50	1.50
Nutmeg powder	0.50	0.50	0.50
Garlic Powder	1.50	1.50	1.50
Ground Black Pepper	0.20	0.20	0.20
Smoked Paprika	2.00	2.00	2.00

phase was collected containing the lipid fraction in trichloromethane. The samples were dried at 80°C until the solvent is completely evaporated and then weighed. The analyses were done in triplicate and lipid content (%) was calculated by Eq. 5.

$$Lipid\% = \frac{p\ x\ 4}{p1}\ x\ 100 \tag{5}$$

Where p represents the sample weight after extraction and drying and p_1 is the weight of the sample collected for analyses, both in grams.

2.11. Total protein determination

The Kjeldahl method was employed to determine protein concentration by converting total nitrogen with a 6.25-factor. Samples were weighed (0.5-0.8 g) and transferred to a Kjeldahl flask with 0.5g of catalyst (selenium dioxide, copper sulfate pentahydrate, and potassium sulfate) and 10 mL of H_2SO_4. The flask was taken to the digestor and the digestion temperature ranged slowly from 50 to 300°C. After four hours, samples became either colorless or mildly green. After cooling, samples were added 20 mL of distilled water and 1 mL of phenolphthalein (1%) and distilled up to 100 mL in a flask containing 10 mL of boric acid and 2-4 drops of methyl red. Samples were titrated with HCl 0.1 M and the analyses were performed in triplicate. The protein fraction was calculated by Eq. 6.

$$Protein_{total} = \frac{V_A \times fa\ x\ 0,14}{p}\ x\ 100 \tag{6}$$

Where V_A represents the volume of titrant (mL), f_a is the correction factor for HCl solution (0.1N), and p is the weight of the sample in grams.

2.12. Total carbohydrate determination

Total carbohydrates were determined by deduction as in Eq. 7.

$$C(\%) = 100-(H + A + P + L) \tag{7}$$

Where C represents carbohydrate ratio (%), H represents humidity, A ash ratio, P total protein ratio, and L lipid ratio, all in %.

2.13. Determination of caloric value

The determination of caloric value for vegetable-sourced sausages was estimated by Eq. 8.

$$Cv = (Px4)+(Cx4)+(Lx9) \tag{8}$$

Where Cv represents the caloric value (kcal.100g^{-1}).

2.14. Determination of sodium, potassium, and calcium content

Quantification of sodium, potassium, and calcium in meat products encompasses two basic steps. The first comprises the preparation of food samples for analysis and the second is the determination of the analytes themselves. Inorganic ash was obtained from 10.0 g of samples at 500°C in a muffle furnace. Ash was dissolved in a strong acid solution and the suspension was filtered. The sample was completed to 100 mL in deionized water and quantification analyses were carried out by flame spectrophotometry (Benfer BFC 150) with standards at 100 mEq, 10 mEq, and 4.0 mEq for Na^+, K^+, and Ca^{2+} respectively. Results were converted to grams per 100 grams. The analyses were performed in triplicate.

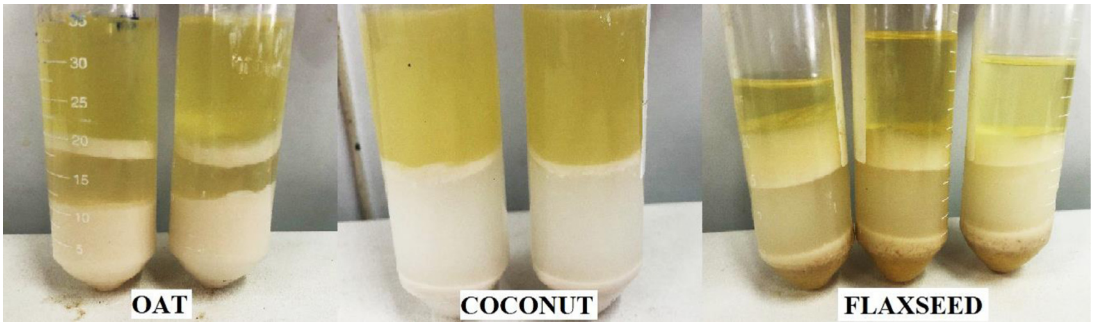

Fig. 1. Emulsion stability of vegetable-sourced flours.

Table 3
Emulsifying activity and emulsion stability of flour samples with standard deviation.

Flour source	Emulsifying activity (%)	Emulsion stability (%)
Oat	41.23 ± 0.85[a]	34.51 ± 0.69[a]
Coconut	56.76 ± 0.65[b]	10.63 ± 0.94[b]
Flaxseed	54.01 ± 0.78[b]	62.66 ± 2.27[c]

[(a, b, c)] Values in a column with the different superscript letters are significantly different ($p < 0.05$).

2.15. Microbiological analyses

The microbiological investigation was accomplished by a certified laboratory (Eurofins, Recife, Brazil) for the control and vegetable-based frankfurter sausages. Microorganisms that indicate food quality and safety were searched and measured: mold and yeast count (ISO 21527-1 2008), total coliform count, and *Escherichia coli* (ISO 4832 2006, ISO 16649-2 2001), and coagulase-positive *Staphylococcus aureus* (ISO 6888-1 1999). Samples were prepared in agreement with ISO 6887-1 (ISO 6887 2017). Sampling was carried out in 0, 60, and 120 days during the storage period.

2.16. Statistics

The data were evaluated according to variance analyses. Mean values and standard deviation were calculated for quantitative analyses and the Shapiro-Wilk test was applied to examine the normality of the data. One-way ANOVA and Tukey's HSD test aimed to find any significant differences among samples. Nonparametric variables were analyzed by the Kruskal-Wallis test. All the statistics were performed with a confidence level of 95% in SPSS® Statistics 26.0.

3. Results and discussion

3.1. Emulsifying capacity and stability of emulsion gels

Three different vegetable-sourced flours were evaluated once their composition comprises favorable indexes of protein and amino acids. These characteristics are important not only because of protein ratios but also to achieve desired texture in the final product, resembling meat sausages which are rich in collagen, fat, and protein. Table 3 displays the results for emulsifying activity and the emulsion stability of samples. These properties are normally determined by the amount and quality of soluble proteins in flour (Santana et al., 2017).

A statistical difference could be observed between the emulsifying activities of oat flour and the flaxseed and coconut flours (for $p < 0.001$). Emulsions of these last two did not differ in activity. Despite CFEG presented the highest emulsifying activity, its emulsion stability was observed to be low which does not feasible its commercial use (Fig. 1).

Fig. 2. Emulsion gels from flaxseed (left) and oat (right) flours at 20% of concentration.

According to Santana et al. (2017), low emulsion stability is inappropriate to subject samples to thermal treatments that are performed in these products.

The gel production causes a tridimensional net of carbohydrates modified or not by thermal processes to be formed with protein and partially denatured lipids (Adebowale and Lawal, 2003; Santana et al., 2017). In this way, it was studied the ability of gel formation from the vegetable-sourced flours at concentrations of 5 and 20%. The alginate-based gelling agent was employed in order to enhance gel stability.

At lower concentrations (5%), coconut flour was not able to present gel structures even in the presence of gelling agents corroborating its low emulsion stability. Oat and flaxseed flour developed weak gels with poor resistance. However, at concentrations of 20%, both samples were observed to result in highly resistant gels. Thus, vegetable-sourced frankfurters were formulated with these two ingredients for future investigation because the stronger the emulsion gel is, the better the texture of the sausages (Fig. 2).

According to Santana et al. (2017), oat flour samples were able to achieve weak EG at 2% of concentration and firm ones at 6%. The weak gel was also observed for flaxseed flour emulsion with flour at a concentration of 10%, although resistant gel structure could be observed at 18%. It is worth mentioning that the resistance of gel may vary according to the intrinsic characteristics of the flour samples.

Vegetable oils are commonly incorporated into food products as a replacement for fat in the form of pre-emulsions mixed with proteins and/or polysaccharides (Zhang et al., 2022). In the emulsification, protein and polysaccharides are adsorbed over the water-oil interface to create a viscoelastic film able to protect oil drops from collision and oxidation, enhancing the stability of the final products. In addition, Baune et al. (2021) found out that the production of stable emulsion

Table 4
Centesnimal composition, color and texture parameters for the vegetable-sourced sausage formulations and the control meat sausage (mean ± standard deviation).

Composition parameter	FFEG Sausage	OFEG Sausage	Control meat Sausage
Humidity (%)	63.08 ± 0.06[a]	67.57 ± 2.29[a]	53.57 ± 0.68[a]
Ash (%)	3.36 ± 0.14[a]	2.85 ± 0.06[a]	3.38 ± 0.27[a]
Sodium (g/100g)	2.62 ± 0.06[a]	2.75 ± 0.59[a]	4.45 ± 0.11[b]
Potassium (g/100g)	1.47 ± 0.05[a]	1.33 ± 0.30[a]	1.39 ± 0.01[a]
Calcium (g/100g)	0.56 ± 0.02[a]	0.55 ± 0.11[a]	0.59 ± 0.08[a]
Total protein (%)	8.79 ± 0.09[a]	2.33 ± 0.05[b]	16.47 ± 0.48[c]
Total lipid (%)	10.05 ± 0.28[a]	12.51 ± 1.15[a]	23.38 ± 0.61[b]
Carbohydrate (%)	14.72 ± 0.11[a]	14.74 ± 2.99[a]	3.57 ± 1.56[b]
Calorific value (kcal/100 g)	184.50 ± 0.02[a]	180.90 ± 0.08[a]	287.00 ± 0.01[b]
Samples/Parameters*			
L*	60.79 ± 0.48[a]	66.31 ± 0.31[b]	51.73 ± 0.45[c]
a*	6.46 ± 0.12[a]	5.25 ± 0.05[b]	4.17 ± 0.13[c]
b*	25.20 ± 0.51[a]	27.20 ± 0.55[b]	16.59 ± 0.52[c]
SF (N m^{-2})	2.46 ± 0.79[a]	2.37 ± 0.56[a]	6.46 ± 1.10[b]

[(a, b, c)] Values in a row with the different superscript letters are significantly different ($p < 0.05$).
[*] Color = [(L*) bright, (a*) redness, (b*) yellowness)]; Texture = [Shear Force, SF)].

gels depends on the capacity of proteins to aggregate themselves, to allow drops to aggregate, and to stabilize the interface between emulsion drops by forming thin but resistant films out of continuous protein. As reported by the results, oat and flaxseed flours could be, therefore, employed in the production of emulsion gels since their characteristics are favorable to add functional and technological properties for a range of applications of interest for the industry.

3.2. Nutritional properties

Table 4 displays the composition of control and vegetable-sourced frankfurters.

Humidity levels were observed to be a little higher for vegetable-sourced sausages, but not statistically different. Similar results were observed for the ash content with no significant difference when compared to the control samples. Protein and lipid ratios were found to be statistically different from the control samples exhibiting considerable reduction for both parameters in vegetable-sourced products.

To adjust the large difference in protein profiles, soya protein and emulsion gel concentrations were elevated. The lipid ratio did not vary significantly between the two vegetable flour formulations. Meat sausages exhibit protein ratios larger than 12% on average. OFEG frankfurter samples displayed considerably lower protein percentages while FFEG frankfurter was found to be 9% of protein. Both vegetable-sourced sausages did not present compatible values with the control sample regarding protein and these results may be related to the absence of animal-sourced raw ingredients such as collagen, which is essential in obtaining minimum protein ratios in the final product. According to Konig (2019), meeting protein targets plays a big role, especially in strict vegan diets, so it is important to achieve protein-rich vegan food products to offer significant nutritional value.

Zhang et al. (2022) reported the replacement of animal-sourced fat by oleo-gel at 25%, 50%, and 100% resulted in a significant increase in monounsaturated and polyunsaturated fat ratios as well as a decrease in saturated fat content in meat products. Fat ratios in vegetable-sourced sausages are significantly inferior to those in control meat sausages (that may vary between 20-45%) as in Table 4. These relatively low fat ratios are related to the higher content of unsaturated fatty acids from vegetable-sourced raw materials and as a consequence, vegetable-sourced sausages are a promising alternative to the food industry fitness sector.

Regarding carbohydrate content, a four-time raise in percentage was observed in the vegetable-sourced frankfurter samples with significant statistical differences. Calorific values displayed a reduction from approximately 40% in vegetable-sourced sausages, therefore, results differ statistically. Between FFEG and OFEG carbohydrate ratios and calorific values, no statistical difference could be observed.

In analyses of the main minerals in control and vegetable-sourced sausage samples, no statistical difference was observed for K and Ca. However, different amounts of Na were attained as can be seen in Table 4.

Sodium is the most important mineral to be evaluated in food products since the consumption of high doses of this mineral is related to heart diseases such as hypertension and cerebrovascular accident. Meat sausages normally present large sodium rates from the several ingredients of the formulation and that contribute to technological properties of the final products such as texture, flavor, and extension of shelf-life (Messias, 2016). There is, in fact, a significant difference in sodium ratio between control and vegetable-sourced samples, and the higher ratios in meat sausages could be associated with animal-sourced raw materials.

3.3. Physical-chemical properties

Color is one of the main reasons consumers decide on food products, especially meat derivatives. It is therefore essential to evaluate color aspects in the development of emulsion gels and the final products (Herrero et al., 2018). According to Munoz-González et al. (2019), emulsion gels from alginate samples are commonly clearer than gels from other gelling agents, such as transglutaminase and gelatin.

Table 4 shows the results for color and texture parameters of vegetable-sourced sausages. They both presented similar colors, however with significant differences regarding redness (a*) and yellowness (b*) when compared to the control samples, which may be related to the dark tones from some spices such as paprika.

In order to look attractive to consumers, meat-type vegan sausages should present a color closer to the red character than brown. The higher L*, a*, and b*, the more red character the products will be. As can be seen in Table 4, the increase in color parameters for vegetable-sourced sausages compared to control meat samples may cause a positive effect due to the use of vegetable flour and improve the acceptability of the consumers.

Similar results were found by Camara et al. (2020) who found that in several tests of emulsion gels, using WHEY, as a fat substitute in meat products, higher L* values showed a reddish color, which may have a positive impact on the final product. In studies with emulsion gels containing oat flour, olive oil and gelling agents (alginate or gelatin), Pintado et al. (2016) observed an increase in a* values and negative b* values depending on the proportion of oat flour used. Increasing redness with the addition of oat bran may be related to a higher concentration of β-glucans, which are reported to confer a darker colour and greater redness in certain bakery end-products. The decrease in b* could be attributed the yellowishgreen hue of olive oil.

In meat products, different textures are commonly associated with some aspects of the formula, such as protein ratio and functionality and ratio and composition of lipids (Estévez et al., 2005; Messias, 2016). The vegetable-sourced samples required lower shear force to the cutting than animal-sourced samples with a significant statistical difference (Table 4). Lipid ratio effects on the texture of sausages have already been reported by Candogan and Kolsarici (2003), who observed that firmness is directly proportional to the fat content. These results are in agreement with the lipid determination since vegetable-sourced sausages were demonstrated to be less firm due to reduced fat content.

Barbut et al. (2016) found the firmness of sausages from canola oil, ethyl cellulose, and sorbitan monostearate to be inferior to sausages from beef fat. According to Baune et al., 2022, increasing the replacement ratio from animal-sourced fat to vegetable-sourced ones causes the firmness and chewiness of sausages to reduce due to the weaker interactions between plant and myofibrillar proteins. As consequence, the final products present a less stable form, and the challenge to manufacture 100% meat-free sausages arises.

In the case of pH, all sausage samples presented acidic values ranging from 5.24 and 5.64, and no statistical difference among each other. Reasonable stability was observed after 120 days of storage in pH values, which corroborates the findings by Konig (2019), which stated that thermally-treated acidic vegan sausages are less prone to microbiological deterioration.

Camara et al. (2021) found similar results to the ones presented when the pH ranged from 5.49 to 6.07. These data are probably related to sodium alginate and they are acceptable to these types of meat products according to other studies, such as Alves et al. (2017) and Paglarini et al. (2019), who investigated low-fat Bologna sausages.

Since water activity plays a key role in the microbiological deterioration of food products, it is of primary importance to attain low values of a_w to keep sausage samples preserved along the storage. Vegetable-sourced frankfurters displayed water activity around 0.97 while mean values for the traditional Portuguese sausages are between 0.60 and 0.95, exhibiting, therefore, a statistical difference and indicating the FFEG and GFEG sausages are more prone to microbial proliferation. Nevertheless, vegetable-sourced frankfurters did not exhibit significant alteration in a_w after 120 days. These results point out the physical stability of the products at the end of storage.

Camara et al. (2021) also observed superior aw values although they did not present statistical significance than control samples. Lower values in control meat sausages may be associated with the higher phosphate concentration once the higher the solute concentration in meat emulsion, the lower the water vapor pressure, and therefore, less water is available for chemical reactions.

3.4. Microbiological analyses

Microbiological assays enabled the identification of the alterations caused in the product throughout time by the number and type of microorganisms related to quality (New Zealand Food Safety Authority 2005). To determine microbial load and the presence of pathogens in food, there are two main groups of methods: the rapid microbial methods and the traditional microbial methods, these last ones normally comprise long-period analyses (Coelho, 2015; Forsythe, 2020).

In this work, a traditional method to investigate microbial growth in a short period was employed. The presence and absence of microorganisms were observed in samples at 0, 60, and 120 days of storage. The average shelf-life of sausage products is 60 days and the storage was extended to evaluate the behavior of parameters for longer periods.

Guidelines for microbiological control standards of cooked food products with the addition of raw ingredients were following the Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA) (Santos et al., 2005).

Regarding yeast, no significant changes were observed during storage and the results met the acceptable control levels for consumption (maximum of 10^4 CFU/g). A significant increase in mold concentration was observed in OFEG sausage samples after 120 days, exceeding the acceptable limits for eating.

By the analytical techniques applied, the investigation demonstrated that the vegetable-sourced sausages did not exhibit detectable levels of thermotolerant coliforms, *E. coli*, and coagulase-positive *Staphylococci* during storage. Thermotolerant coliforms were observed to increase in meat sausage samples after 60 days, but the results were still in accordance with the acceptable limits for consumption (maximum of 10^2 CFU/g).

As could be observed from the microbiological analyses, the vegetable-sourced frankfurter-type sausages presented satisfactory levels of food quality and they comply with the Portuguese legislation (Regulation (CE) n.° 1441/2007), lasting up to 120 days.

Sensory analyses may be useful to control and record significant temporal alterations in food, therefore, important to estimate shelf-life. To obtain accurate results, the sensory analysis should be performed under typical storage and consumption conditions (New Zealand Food Safety Authority 2005). However, it was not possible to carry out these analyses at the present moment of the study (2020-2022) due to the Covid-19 pandemic. That is because the investigation should comprise consumers and trained experts in a controlled environment, which has become unattainable in these years.

4. Conclusions

It was possible to conclude that flaxseed and oat flours presented great emulsion capacities and efficiency in producing emulsion gels. The emulsion gels were used to replace animal-sourced protein and fat in meat-type food in order to add positive effects to the composition of sausages. Despite the substitution of animal-sourced ingredients causing changes in the final product characteristics, these changes could be considered acceptable according to nutritional, physical-chemical, and microbiological quality parameters.

As a future perspective, it would be the study of new formulations that improve the texture and consequently decrease the water activity of the vegan sausages so that they come closer to the meat analogs. And the realization of sensory analysis would be a next step, as they are important in determining the shelf life of the product and consumer acceptability.

CRediT author statement

All authors contributed to the article and approved the submitted version.

Informed consent

Written informed consent was obtained from all study participants.

Declaration of Competing Interest

Declaration of Interest Statement: The authors declare that they do not have any conflict of interest.

CRediT authorship contribution statement

Priscilla Ferreira Corrêa: Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Carla Fabiana da Silva:** Formal analysis. **João Paulo Ferreira:** Writing – review & editing. **Jenyffer Medeiros Campos**

Guerra: Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

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