

ORIGINAL ARTICLE

Plant based proteins as an egg alternative in cookies: using de-oiled sunflower meal and its protein isolate as an emulsifying agent

Proteínas vegetais como alternativa ao ovo em biscoitos: uso de farinha de girassol sem óleo e sua proteína isolada como um agente emulsionante

Thais Dolfini Alexandrino¹, Elizabeth Harumi Nabeshima¹ , Nathália do Amaral Gastardo¹, Mitie Sônia Sadahira¹, Isabel Muranyi², Peter Eisner², Maria Teresa Bertoldo Pacheco^{1*} 

¹Instituto de Tecnologia de Alimentos (ITAL), Campinas/SP - Brasil

²Fraunhofer Institute for Process Engineering and Packaging IVV (Fraunhofer IVV), Freising - Germany

*Corresponding Author: Maria Teresa Bertoldo Pacheco, Instituto de Tecnologia de Alimentos (ITAL), Av. Brasil, 2880, CEP: 13070-178, Campinas/SP - Brasil, e-mail: mtb@ital.sp.gov.br

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Abstract

De-oiled sunflower meal (DSF) and its protein isolate were evaluated as emulsifiers to replace egg yolk powder (EYP) in cookies. The chemical emulsifier DATEM (Diacetyl Tartaric Acid Esters of Mono- and Diglycerides) was used as a positive control. An experimental design of mixtures of the simplex-centroid type was carried out, and the ingredients were expressed as pseudo-components for EYP, DSF, and DATEM emulsifier. The DSF and its sunflower protein isolate (SPI) were tested to validate the design in optimized conditions. Whole meal cookies were analyzed in relation to rheological, physical, technological, and sensory characteristics using the control difference test. In the rheology of the dough, the DSF caused a reduction in the value of hardness, while increasing the parameter of elasticity. Instrumental texture results as well as the specific volume of the samples showed no difference. The control difference test regarding the cookies made with EYP, SPI, and DSF showed that consumers did not give different ratings to cookies made with sunflower as an emulsifier. Therefore, according to the parameters, conditions, and analysis performed, the replacement of EYP by DSF and SPI proved to be satisfactory as an emulsifying agent regarding the preparation of cookies for vegan consumers.

Keywords: Sunflower protein isolate; Emulsifier; Vegan cookies; Rheological profile; Emulsifying capacity; Egg substitute; Experimental design.

Resumo

A farinha de girassol desengordurada (DSF) e seu isolado proteico foram avaliados como emulsificantes para substituir a gema de ovo em pó (EYP) em biscoitos. O emulsificante químico DATEM (Ésteres de Ácido Diacetil Tartárico de Mono e Diglicerídeos) foi utilizado como controle positivo. Foi realizado um planejamento experimental



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de misturas do tipo simplex-centróide, e os ingredientes foram expressos como pseudocomponentes para o emulsificante EYP, DSF e DATEM. O DSF e seu isolado de proteína de girassol (SPI) foram testados para validar o projeto em condições otimizadas. Biscoitos integrais foram analisados quanto às características reológicas, físicas, tecnológicas e sensoriais por meio do teste de diferença de controle. Na reologia da massa, o DSF provocou redução no valor da dureza, ao mesmo tempo em que aumentou o parâmetro de elasticidade. Os resultados de textura instrumental, bem como o volume específico das amostras, não mostraram diferença. O teste de diferença de controle em relação aos biscoitos feitos com EYP, SPI e DSF mostrou que os consumidores não deram notas diferentes aos biscoitos feitos com girassol como emulsificante. Portanto, de acordo com os parâmetros, condições e análises realizadas, a substituição do EYP por DSF e SPI mostrou-se satisfatória como agente emulsificante no preparo de biscoitos para consumidores veganos.

Palavras-chave: Proteína isolada de girassol; Emulsificante; Biscoitos veganos; Perfil reológico; Capacidade emulsificante; Substituto de ovo; Design experimental.

Highlights

- Sunflower by-product has high technological performance as an emulsifier
- Potential of sunflower meal as a technological ingredient in whole meal cookies
- Egg replacement by sunflower protein in vegan cookies

1 Introduction

Consumers with different dietary restrictions or preferences have driven the food market toward new product options, ingredients and food additives (Asioli et al., 2017). Among them, there are intolerant or allergic consumers, vegetarians and vegans. While vegans seek to exclude all ingredients of animal origin from their diet (Saari et al., 2020), individuals with food intolerances and allergies need to exclude some foods due to their allergenicity (Food Allergy Research & Education, 2018). Both categories mentioned above, restrict the consumption of bakery products, which widely use milk, whole egg, or yolk in their formulation. The egg performs several technological functions in the dough, and the yolk is considered an excellent emulsifying agent (Arozarena et al., 2001).

Emulsifiers represent an important resource to improve the technological quality of whole-grain bakery products due to a series of benefits that confer the dough, which range from greater ease of handling the dough to increases in volume and texture throughout the shelf life of the dough (Ribotta et al., 2004; Gandra et al., 2008). This functional improvement occurs through the formation of complexes with starch and modification of the rheological properties of wheat meal by interaction with gluten. There is an improvement in the consistency and texture of fat-based products, as well as the promotion of the solubilization of aromas (Barros et al., 2007).

In this context, proteins from sunflower have great potential for use as an emulsifier (González-Pérez & Vereijken, 2007; Wildermuth et al., 2016), due to their ability to produce stable emulsions with values comparable to commercial plant proteins, such as soybean (Salgado et al., 2012). Other attractive aspects for the use of sunflower as a food ingredient are that it is not a genetically modified organism (GMO) and rarely presents allergenicity (Gassmann, 1983; González-Pérez & Vereijken, 2007; Wildermuth et al., 2016).

Compared to soy protein, which already has a high and well-established commercial value, sunflower protein needs some improvements as it has a high content of phenolic compounds and fibers from the husks, when using conventional oil extraction (González-Pérez et al., 2002; United States Department of Agriculture, 2018). However, new alternatives have been applied, such as removing husks before oil

extraction, as well as removing polyphenols afterward. Some studies have been showing that processing with peeled seeds allows an interesting variety of ingredients for application in several food products (Alexandrino et al., 2017; González-Pérez et al., 2002; Pickardt et al., 2009; Salgado et al., 2011).

De-oiled sunflower meal has a high protein content and appropriate amino acid profile for use in the human diet (Alexandrino et al., 2017). In addition to the nutritional aspect, proteins are classically used as ingredients due to their technological functional qualities. Sunflower proteins applied in cookies and noodles or in bread used as a substitute up to 10% instead of wheat meal, showed good acceptability by consumers, in addition to increasing the nutritional value of the products (Bhise et al., 2014, 2015; Shchekoldina & Aider, 2014). In view of the above scenario, the objective of this study was to evaluate cookie formulation added with de-oiled sunflower meal (DSF) and its sunflower protein isolate (SPI) reduced in phenolic as an emulsifier to replace egg yolk.

2 Materials and methods

2.1 Ingredients

DSF was obtained from the Fraunhofer Institute IVV (Freising, Germany) and presented the following specification: 59.6% of protein, 8.7% of fiber, 10.1% of minerals and 2.9% of fat and for functional properties: 38% of protein solubility, 530 ml/g emulsifying capacity, 874% of foaming capacity and 87% of foaming stability. SPI was prepared according to Alexandrino et al. (2017), which showed 92.0% of protein without phenolic compounds. Wheat meal for baking was supplied by J. Macêdo S/A (Ceará, Brazil) and whole wheat meal supplied by SL Alimentos Ltda (Paraná, Brazil) was used. Egg yolk powder (EYP) was supplied by Shinoda Alimentos (São Paulo, Brazil) and DATEM emulsifier (Mono and diglyceride diacetyl tartrate) by Granotec/ Granolab do Brasil S/A. The other ingredients were purchased at the local market.

2.2 Chemical characterization of DSF, SPI and cookies

DSF, SPI and cookies were analyzed for moisture content (AOAC, method no.925.09), whereas ash (AOAC, method no.923.03), dietary fiber (AOAC, method no. 985.29), and protein, by the Kjeldahl method, using a conversion factor of 5.75 (AOAC, method no.960.52), were performed according to Latimer Junior (2012). The lipid content (AOCS Ai 3-75) according to Firestone (2013). The water activity of cookies was performed in triplicate, on the 7th day after processing, with the AquaLab digital device model 4TEV (Decagon, Pullman, USA), according to the manufacturer's instructions (Decagon Devices, 2015).

2.3 Emulsifying capacity of ingredients

The emulsifying capacity of EYP, DSF, and SPI was performed according to Pilosof (2000) with modifications, being the verification of the visual break of the emulsion inversion point. Samples at 0.1% (w/v) were dispersed in distilled water for 45 s at 8000 rpm and homogenized in ULTRATURRAX T25 (IKA-Werke GmbH & Co., Germany). Then, the samples were followed with the addition of sunflower oil (Suavit®) slowly under continuous stirring at 9500 rpm of the homogenizer. Results were displayed as mL of oil emulsified by one g of sample.

2.3.1 Experimental design

A simplex-centroid design was used for mixtures of three components, with three repetitions of the central point (Table 1) for the preparation of cookies. The variables analyzed were: reconstituted EYP, DSF solution, and DATEM emulsifier (E). EYP reconstitution was performed according to the manufacturer's

recommendation, while the preparation of DSF aqueous solution was calculated for 20% of protein according to Arozarena et al. (2001). In addition in the formulations, the same percentages were used for EYP and DSF, 5.8% of proteins.

To calculate the independent variables levels, the lower and upper limits were considered, which for EYP and DSF ranged from zero to 5.8% of proteins and for variable E ranged from zero to 1%. The sum of the proportions of the components was equal to 1 and the content of these variables was calculated in percentage terms, considering for EYP and DSF that 1 = 5.8 g/100 and for E that 1 = 1 g/100 g.

Table 1. Simplex-centroid design of mixtures with coded and real values.

| Ingredient proportions in the ternary mixture | | | | | | |
|---|--------------------------------|----------------|----------------|------------------------|------|-------|
| Tests | Proportion in pseudo-component | | | Concentration used (%) | | |
| | X ₁ | X ₂ | X ₃ | EYP | DSF | E |
| 1 | 1 | 0 | 0 | 5.8 | 0 | 0 |
| 2 | 0 | 1 | 0 | 0 | 5.8 | 0 |
| 3 | 0 | 0 | 1 | 0 | 0 | 1 |
| 4 | 0.5 | 0.5 | 0 | 2.9 | 2.9 | 0 |
| 5 | 0.5 | 0 | 0.5 | 2.9 | 0 | 0.5 |
| 6 | 0 | 0.5 | 0.5 | 0 | 2.9 | 0.5 |
| 7 | 0.666 | 0.167 | 0.167 | 3.86 | 0.97 | 0.167 |
| 8 | 0.167 | 0.666 | 0.167 | 0.97 | 3.86 | 0.167 |
| 9 | 0.167 | 0.167 | 0.666 | 0.97 | 0.97 | 0.666 |
| 10 | 0.333 | 0.333 | 0.333 | 1.93 | 1.93 | 0.33 |
| 11 | 0.333 | 0.333 | 0.333 | 1.93 | 1.93 | 0.33 |
| 12 | 0.333 | 0.333 | 0.333 | 1.93 | 1.93 | 0.33 |

EYP = reconstituted egg yolk powder. DSF = de-oiled sunflower meal, E= DATEM emulsifier.

2.4 Cookie production

The formulation was performed according to Clerici et al. (2013), presented in Table 2.

Table 2. Control cookie formulations containing DSF, SPI, and emulsifier.

| Ingredients | Control | |
|-------------------------|----------------|------------|
| | % ^A | Amount (g) |
| Meal | 100 | 270 |
| Whole meal | - | - |
| DSF or SPI* | - | - |
| EYP (protein content) | 5.8 | 15.6 |
| DATEM emulsifier* | - | - |
| Refined sugar | 33 | 90 |
| Glucose syrup | 3.7 | 10 |
| Condiments ^B | 0.1 | 0.5 |
| Salt | 0.4 | 1 |
| Chemical leavening | 1.5 | 4 |
| Fat | 23 | 62.5 |
| Water | 26 | 71.4 |
| Ammonium bicarbonate | 0.9 | 2.3 |

^APercentage of ingredients were calculated based on 100% of whole wheat and refined meal. ^BCondiments = clove, cinnamon, and nutmeg powder. * % EYP or DSF or SPI varied according to experimental design. EYP = reconstituted egg yolk powder. DSF = de-oiled sunflower meal. SPI = sunflower protein isolated.

2.5 Cookie preparation

The cookie was prepared according to the two-phase creaming method (Manley, 1983). Briefly, the yolk reconstitution was according to manufacturer's recommendation using the 1:1.2 ratio to EYP and water, and to disperse the DSF and SPI were used 1 to 2.38 of water ratio, to each sunflower derivative type. These solutions resulted in 5.8% of protein, that were homogenized with water for 60 s in a planetary mixer (KitchenAid, model K5SS). After, it was added the sugars and water to prepare the cream phase. Then, refined and whole wheat meal, salt, seasonings, and yeast were added, homogenized for 1.5 min, at low speed, level 2 (96 rpm), finally receiving the rest of the water until the dough stay cohesive. The dough was laminated (8 mm), cut (3.5 cm in diameter), and baked (convection oven, Perfecta) for 9 min at 165 °C. After cooling, the products were packed in high-density laminated packaging and stored at room temperature for further analysis.

2.6 Rheological profile of cookie dough

The dough properties of refined and whole wheat meal mixtures (50:50) and different levels of yolk powder, sunflower meal and emulsifier (experimental design in Table 2) were evaluated according to Fustier et al. (2008), through the TPA method (texture profile analysis), using the Stable Micro Systems Texture Analyzer TAXT2i texturometer. Cylindrical dough discs, 25 mm in diameter and 20 mm in thickness, were prepared with an aluminum circular shaper. The parameters used were: probe P100 (100 mm compression platen), pre- and post-test speed 2 mm/s, test speed 0.8 mm/s, compression of 40% of the original thickness, and waiting period between strokes 5 s.

2.7 Physical characteristics of cookies

2.7.1 Specific volume, instrumental texture, and color

The specific volume of the cookies was evaluated by the method of displacement of rapeseeds according to method 10.05.01 of the American Association of Cereal Chemists International (2010), using a volume measuring cup.

The texture properties of the cookies were analyzed instrumentally several times within 7 days after processing. The hardness of the cookies was determined using the Stable Micro Systems Texture Analyzer TAXT2 texturometer, 3-Point bending rig probe (HDP/3PB), and HDP/90 platform. The results were expressed in N. The parameters used in the tests were: pre-test speed = 1.0 mm/s; test speed = 3.0 mm/s; post-test speed = 10.0 mm/s; distance 5 mm, with compression force measurement.

The color was determined in triplicate by the CIE L*a*b* color system using a CR 450 Konika Minolta Chroma Meter portable colorimeter (Minolta Chroma Co., Osaka, Japan).

2.8 Optimized condition validation

Three formulations were processed: control sample EYP, DSF, and sample containing SPI, according to formulation in Table 1.

2.9 Sensory evaluation of cookies

Thirty trained sensory panelists evaluated the global difference between the control sample (with EYP) and the samples containing DSF and SPI according to the "Difference from Control" test (Meilgaard et al., 2015). The cookies assays were presented coded with three-digit numbers and served at room temperature (25 ± 2 °C). The tasters were asked to assign scores to each sample according to the degree of global difference regarding to the control. They were defined as differences between parameters and included the

global flavor, color, texture, and other attributes that the panelists considered relevant, using a nine-point scale anchored by the terms “extremely less or inferior” for grade 1 and “extremely more or superior” for grade 9. The samples were presented in complete randomized blocks, in a monadic way (Stone & Sidel, 1993). Before starting the tests, a “Free and Clarified Term of Consent approved by the Ethics Committee” (number CAAE: 58901516.9.0000.5374) was signed by everyone.

2.10 Statistical analysis

Results were analyzed by Analysis of Variance (ANOVA) with Tukey’s post-hoc test using a significance level of 5%. ANOVA was also applied in the construction of models and contour graphs for the analysis of mixture designs. For both types of analysis, Statistica software version 10.7 was used.

3 Results and discussion

3.1 Emulsifying capacity and chemical composition of EYP, DSF and SPI

The emulsifying capacity of EYP (873.88 mL oil/ g) was the highest followed by SPI with 815.19 mL oil/ g and DSF with 568.75 mL oil/ g. The values were higher compared to mung bean protein isolate with 245 mL oil/ g and slightly lower compared to mesquite seeds protein isolate with 913 mL oil/g (El-Adawy, 2000; Silva et al., 1997)

DSF presents stable emulsions in acid pH, while alkaline and neutral pH values can lead to precipitation and protein aggregation, which affects emulsion stability. Due to the functional properties observed, the sunflower proteins should be used in emulsions with preferably acid pH values (González-Pérez et al., 2005). When considering a dry basis, the DSF and SPI samples presented a chlorogenic acid content (ACG) of 2.46 and 0.45 g/100 g, respectively (Alexandrino et al., 2017).

3.2. Dough Rheology and technological properties of cookies using experimental mixture design

Rheology results of the cookie dough obtained from different mixtures, as indicated by the experimental design are presented in Table 3. The parameters analyzed were a_w , consistency, hardness, cohesiveness and elasticity of the dough. The equations and determination coefficients of the models obtained are shown in Table 4. All the mathematical models generated presented determination coefficients from 75.06 to 97.49%, indicating that the equations obtained can be used as trend or prediction models.

Rheological analyzes are used to monitor the dimension of the cookies (volume and weight), because depending on the ingredient added, a loss of balance between the weight, diametrical and longitudinal volume of the products can occur (Fustier et al., 2008). In addition, it should be considered that DATEM as well as egg yolk lecithin emulsifiers, contribute to the machinability and lamination of cookie dough, and their replacement by sunflower meal could interfere with these results.

Regarding consistency, the values ranged from 1531.2 gf to 3374.6 gf and the use of DFS interfered with consistency, promoting a decrease in this value (Figure 1A). In contrast, the emulsifier and EYP, at the highest level of addition, presented the highest values of this parameter. For hardness, a variation of 1607.1 gf to 3437.5 gf was obtained. Figure 1B shows that the use of EYP in the range of 0.5 to 1.0 and the use of emulsifier in the range of 0.25 to 1.0 presented the highest values for this parameter. The use of DFS showed higher values within the range from 0.75 to 1.0, with lower values compared to the other two ingredients. Considering cohesiveness, the variation ranged from -0.01 to -0.2, and finally for elasticity, from -0.2 to 266.9. Figure 1C shows that the use of DFS resulted in high values for this parameter, as well as the use in the range of 0 to 0.25 for EYP.

Table 3. Rheological profile of cookie dough according to mixture design.

| Test | Consistency (gf) | Hardness (gf) | Cohesiveness | Elasticity |
|------|------------------|---------------|--------------|------------|
| 1 | 3374.6 | 1740.7 | -0.01 | -0.2 |
| 2 | 1784.9 | 1945.0 | -0.08 | -1.7 |
| 3 | 3436.6 | 3437.5 | -0.03 | -0.9 |
| 4 | 1606.6 | 1607.1 | -0.12 | -1.9 |
| 5 | 2096.6 | 2862.0 | -0.07 | -198.9 |
| 6 | 2117.4 | 2889.0 | -0.09 | -266.9 |
| 7 | 1667.4 | 2235.9 | -0.08 | -179.4 |
| 8 | 1785.2 | 2396.9 | -0.07 | -171.7 |
| 9 | 1531.2 | 2017.9 | -0.22 | -140.3 |
| 10 | 2086.2 | 2884.8 | -0.06 | -164.7 |
| 11 | 2253.6 | 3048.2 | -0.02 | -69.2 |
| 12 | 2195.9 | 2941.4 | -0.03 | -60.7 |

Table 4. Adjusted polynomial model, significance level (p) and coefficient of determination (R) for a_w and rheological properties.

| Variable | Adjusted model | R ² | p |
|-------------|---|----------------|-------|
| a_w | $A_w = +0.289*x + 0.459*y + 0.229*z + 0.214*x*y + 0.6741*x*z + 0.374*y*z - 2.247x*y*z + 0.999*x*y*(x-y) - 0.378*x*z*(x-z)$ | 0.9749 | 0.025 |
| Consistency | $C = +3401.411*x + 1811.642*y + 3463.446*z - 3783.890*x*y - 5128.293*x*z - 1865.124*y*z + 1288.562*x*y*z - 8765.231*x*y*(x-y) + 5116.009*x*z*(x-z)$ | 0.9675 | 0.036 |
| Hardness | $H = +1816.683*x + 2020.869*y + 3513.576*z - 636.596*x*y + 1396.465*x*z + 1096.638*y*z - 5670.481*x*y*z - 6658.102*x*y*(x-y) + 11752.442*x*z*(x-z)$ | 0.7506 | 0.511 |
| Elasticity | $E = +4.136*x + 2.634*y + 3.433*z + 13.309*x*y - 776.086*x*z - 045.620*y*z + 1261.226*x*y*z - 269.606*x*y*(x-y) - 1600.726*x*z*(x-z)$ | 0.9621 | 0.045 |

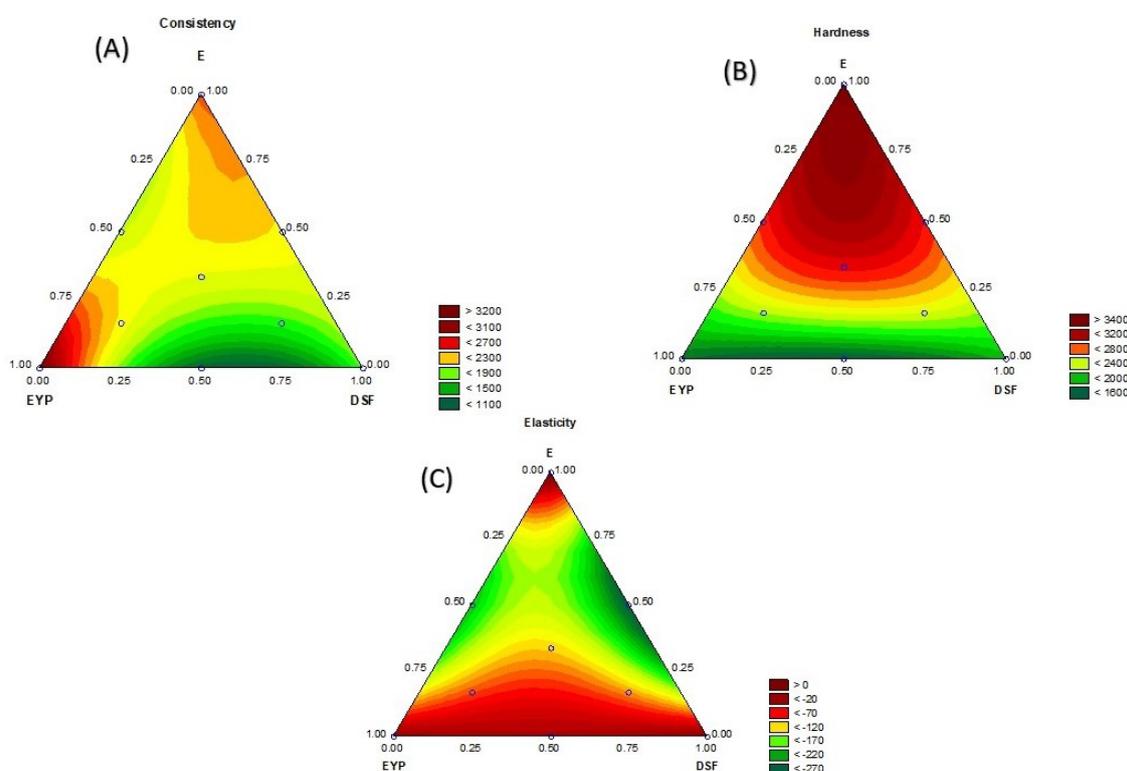


Figure 1. Effect of the addition of reconstituted egg yolk powder (EYP), de-oiled sunflower meal (DFS) and DATEM emulsifier (E) on dough rheology: (A) consistency, (B) hardness and (C) elasticity.

Table 5 shows that the highest consistency value (2.99 g/cm³) was obtained with the highest percentage of DSF (test 8) compared to the other ingredients. The results ranged from 3.49 for test 1 (100% of EYP) to 3.01 (for test 5) which contained 50% of EYP and 50% of E. For the color parameters L*, a* and b*, the variations were from 43.72 to 53.73, 8.79 to 11.6 and 22.6 to 25.64, respectively. This demonstrates that the replacement of EYP and E by DSF and SPI remained unaffected, showing the application potential of sunflower ingredients.

The results of water activity and instrumental texture analyzed in the first week after processing, showed a variation of 0.23 to 0.46 for a_w and of 2105.4 gf to 4130.3 gf regarding hardness, respectively. For this parameter, the highest value was obtained for the test with 100% of DSF (test 2), and the lowest value for test 3, with 100% of E in its formulation. However, test 2 presented the highest value (4130.3 gf) which was similar to cookies containing the mixture of ingredients, as well as test 8 containing higher proportions of DSF (4122.7 gf) and test 9 containing higher proportions of E (4094.2 gf). According to Mamat & Hill (2014), the hardness is correlated with dimension, due to the emulsifier's capacity to stabilize the air cells in the dough structure more homogeneously.

In Figure 2 it is possible to see that the water activity was independent on the cookie formulation for all tests. The highest value was 0.46, which is within the safety limit (a_w < 0.6) for food products regarding microbial stability. For the parameters volume, dimensions, L*, a*, b* and texture, the R² obtained were low, with values: 0.453, 0.629, 0.486, 0.459, 0.501, 0.648, respectively, thus trend models and graphs were not generated outline.

Table 5. Specific volume and dimensions on the processing day, and water activity (a_w) and instrumental texture 7 days after processing, referring to the design of mixtures.

| Assay | Specific volume | | Color | | | a _w | Hardness (gf) |
|-------|----------------------|-----------|-------|--------|-------|----------------|---------------|
| | (cm ³ /g) | Dimension | L* | a* | b* | | |
| 1 | 1.55 | 3.49 | 47.89 | 11.2 | 24.53 | 0.29 | 2431.7 |
| 2 | 1.54 | 3.27 | 53.73 | 8.79 | 25.23 | 0.46 | 4130.3 |
| 3 | 1.24 | 3.32 | 48.94 | 10.24 | 24.05 | 0.23 | 2105.4 |
| 4 | 1.35 | 3.04 | 49.28 | 9.91 | 24.26 | 0.43 | 2855.8 |
| 5 | 1.98 | 3.01 | 52.66 | 9.34 | 24.39 | 0.43 | 3274.7 |
| 6 | 2.25 | 3.23 | 51.25 | 9.79 | 24.60 | 0.44 | 3229.5 |
| 7 | 2.43 | 3.09 | 43.72 | 11.60 | 22.60 | 0.40 | 3959.6 |
| 8 | 2.99 | 3.05 | 48.88 | 10.625 | 24.42 | 0.37 | 4122.7 |
| 9 | 2.78 | 3.09 | 47.44 | 11.26 | 24.19 | 0.37 | 4094.2 |
| 10 | 2.14 | 3.49 | 52.31 | 9.12 | 24.29 | 0.41 | 3295.8 |
| 11 | 1.92 | 3.34 | 49.71 | 10.54 | 24.98 | 0.37 | 3212.1 |
| 12 | 1.77 | 3.21 | 51.13 | 10.24 | 25.64 | 0.38 | 3432.6 |

L*, a*, b* = color parameters.

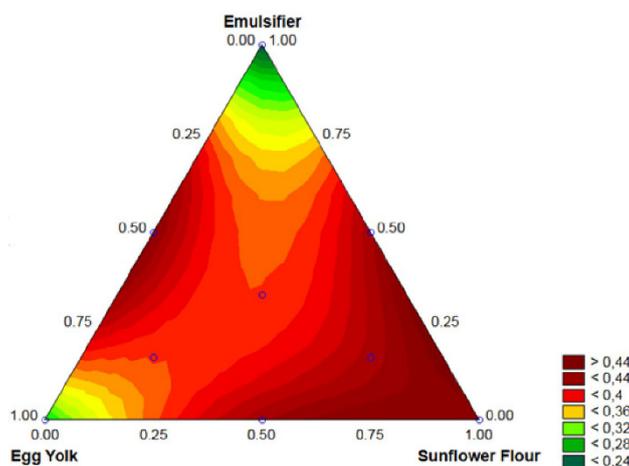


Figure 2. Effect of the addition of reconstituted egg yolk powder (EYP), de-oiled sunflower meal (DSF) dispersion and DATEM emulsifier (E) on water activity.

3.3 Physico-chemical and sensory characteristics of cookie formulations

The cookies prepared with EYP and SPI showed both similar protein and lipids content (Table 6). The results show high potential in sunflower proteins, while simultaneously excluding the use of chemical emulsifier. The cookies' approximate composition makes it possible to attribute the technological and physical characteristics evaluated by the emulsifying properties of the ingredients used in the formulations.

Table 6. Chemical composition of cookies made with egg yolk powder (EYP), sunflower protein isolate (SPI) and de-oiled sunflower meal (DSF).

| Cookies | Chemical composition (%) | | | | |
|---------|-----------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| | Lipids ^A | Proteins ^A | Ashes ^A | Fiber ^A | Carbohydrate ^B |
| EYP | 14.3 ± 0.12 ^b | 8.75 ± 0.06 ^a | 1.58 ± 0.01 ^a | 4.76 ± 0.03 ^a | 69.98 |
| DSF | 15.33 ± 0.09 ^a | 8.28 ± 0.15 ^b | 1.41 ± 0.00 ^b | 4.55 ± 0.05 ^a | 70.43 |
| SPI | 15.00 ± 0.07 ^{a,b} | 8.87 ± 0.02 ^a | 1.44 ± 0.02 ^b | 4.61 ± 0.08 ^a | 70.07 |

^AChemical composition values were expressed on a dry matter basis. Protein (N = 5.75). (n = 2). ^BCarbohydrate content was calculated by difference. ^{a,b}Values in columns followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's post-hoc test.

Technological and physical characteristics of cookies using EYP, SPI, and DFS as emulsifying agents are presented in Table 7. As for the results of instrumental color by the L*a*b* system, it is observed that there was no statistical difference ($p < 0.05$) between cookies, showing that the amount used of these ingredients in the formulation did not interfere with the color of the products. The images of cookies can be seen in Figure 3.

Table 7. Physical characteristics of cookies made with egg yolk powder (EYP), sunflower protein isolate (SPI), and de-oiled sunflower meal (DSF).

| Cookies | L* | a* | b* | Water activity | Specific volume (cm ³ /g) | Hardness (N) |
|---------|-------------------------|--------------------------|-------------------------|--------------------------|--------------------------------------|--------------------------|
| EYP | 48.6 ± 0.4 ^a | 10.4 ± 0.46 ^a | 24.4 ± 0.2 ^a | 0.56 ± 0.00 ^a | 1.5 ± 0.02 ^a | 27.4 ± 3.2 ^c |
| SPI | 47.5 ± 0.8 ^a | 10.7 ± 0.2 ^a | 24.6 ± 0.4 ^a | 0.46 ± 0.00 ^b | 1.4 ± 0.03 ^a | 71.9 ± 2.19 ^a |
| DSF | 47.8 ± 0.9 ^a | 10.4 ± 0.5 ^a | 24.2 ± 0.1 ^a | 0.44 ± 0.03 ^b | 1.4 ± 0.05 ^a | 66.5 ± 3.9 ^b |

^{a,b,c}Values in columns followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's post-hoc test. L*, a*, b* = color parameters.



Figure 3. The visual appearance of cookies made with egg yolk powder (EYP), de-oiled sunflower meal (DSF) and sunflower protein isolate (SPI).

The water activity showed reduced values and small variation between samples (0.44 to 0.56), where the highest value was from the control sample containing EYP and different from the cookies containing sunflower derivatives. According to Clerici et al. (2013), this parameter provides important information about the shelf life of a product and values lower than 0.60 are desirable to obtain more stable products to avoid loss of crispness.

Control cookies had higher specific volume and lower hardness, probably related to the emulsifying capacity of the egg yolk, according to the data presented.

The sensory analysis performed by the control difference test (Table 8) showed similar results to the physical analyses. The color and texture of the control cookies showed lower values, however, significantly different ($p < 0.05$) color, texture and global flavor from the cookies containing sunflower ingredients. Some panelists commented that the texture of cookies with sunflower meal was harder, while cookies with protein isolate as an emulsifying agent were crunchier. The lower values in the texture parameter for the control sample were due to loss of crispness.

The hardness of a solid food is defined as the force required to compress a substance when placed between molar teeth. Mamat & Hill (2014) compared sensory and instrumental methods, in which the first method mentioned assesses the complexity of perception, followed by immediate analysis and results integration, which allowed the interpretation of a large number of unique textural sensations at the same time.

Table 8. Test of difference in the control of cookies made with egg yolk powder (EYP), sunflower protein isolate (SPI) and de-oiled sunflower meal (DSF).

| Cookies | Color | Texture | Global flavor |
|---------|--------------------------|--------------------------|--------------------------|
| EYP | 5.09 ± 0.93 ^b | 5.00 ± 0.95 ^b | 4.75 ± 0.80 ^a |
| SPI | 6.19 ± 1.12 ^a | 7.97 ± 0.86 ^a | 4.91 ± 1.47 ^a |
| DSF | 6.06 ± 0.84 ^a | 7.75 ± 0.76 ^a | 5.06 ± 1.19 ^a |

^{a,b}Values in columns followed by the same letter are not significantly different ($p > 0.05$) according to Tukey's post-hoc test.

Regarding the global flavor, no significant difference ($p > 0.05$) was found between the samples, even though some panelists noticed a slightly bitter residual in the cookies containing sunflower meal. The tasters reported that the samples containing sunflower ingredients were different from the control, but were considered more pleasant.

4 Conclusion

De-oiled sunflower meal (DSF) features important ingredient properties due to its high protein content. In addition, the nutritional protein properties present technological functionality, including emulsification. Due to the higher values of water activity and texture, the replacement of 100% of sunflower meal resulted in a less soft and crunchier cookie. The sensory analysis showed no difference in flavor between the samples prepared with egg yolk powder (EYP) or with the sunflower ingredients. In contrast, color and hardness parameters were rated higher for the cookies containing sunflower ingredients. Therefore, DSF can be used as an emulsifier to replace EYP as an ingredient in the production of vegan cookies and probably in other bakery alternatives. According to the results of this study, it was possible to validate that the sunflower is a plant based alternative food of great nutritional relevance. The study contributes information to the development of products with DSF and sunflower protein isolate, as well as the valorization of the sunflower production chain. Moreover, the importance of sunflower meal in the bakery industry is the possibility to replace animal proteins (eggs) with plant-based proteins, which have functional emulsifying properties.

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