# Chapter 7
# Effect of Partial Replacement of Milk Protein by Vegetable Proteins on the Texture of *Requeijão*

**Alex Paulo Rodrigues, Rafael Resende Maldonado, and Maria Teresa de Alvarenga Freire**

## 7.1 Introduction

According to the Food and Agricultural Organization of the United Nations ( FAO 2016), the dried seeds obtained from leguminous plants are denominated pulses. Singh (2017) remarks that all pulses are considered leguminous, but not all leguminous are considered pulses. The (FAO 2016) states that pulses include kidney beans, lima beans, butter beans and broad beans. Chickpeas, cowpeas, black-eyed peas and pigeon peas are also pulses, as well as all varieties of lentils. Pulses have been considered by several researchers as the second source of human food, being surpassed only by cereals. Their importance is due to the high content of macronutrients such as proteins and carbohydrates; micronutrients such as vitamins and minerals and their low calories and lipid content (Asif et al. 2013; Roy et al. 2010). Their bioactive constituents have been attracting attention and raising the interest of the food industry. Studies report benefits such as reducing blood plasma glucose levels, which aids in the control of obesity, diabetes, lowering serum cholesterol levels, and preventing cardiovascular disease. Furthermore, the peptides found in pulses may present antioxidant, antimicrobial, and antitumor activity (Mudryj et al. 2014).

A. P. Rodrigues · M. T. de Alvarenga Freire (✉)
Departamento de Engenharia de Alimentos, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo Campus Fernando Costa, São Paulo, Brazil
e-mail: freiremt@usp.br

R. R. Maldonado
Departamento de Alimentos, Colégio Técnico de Campinas, Universidade Estadual de Campinas, Campinas, Brazil



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### 7.1.1 Composition

Regarding macronutrients, pulses contain approximately 21–25% protein, almost twice the content found in cereals (Singh 2017). However, they have limited levels of essential amino acids such as methionine, tryptophan and cysteine (Singh 2017; Havemeier et al. 2017). Therefore, it is recommended that pulses are consumed in combination with other sources of vegetal or animal protein to form a good quality protein blend and complement the diet (Asif et al. 2013; Havemeier et al. 2017). The protein content and amino acid composition vary according to the variety, germination, environment and application of fertilizers. Globulins, stock proteins rich in glutamine, aspartic acid, arginine and lysine, correspond to 37–72% of the protein composition. Legumins and vicilins, rich in methionine and cysteine, are the main globulins found in pulse seeds. The complementary fraction consists of albumin, which contributes approximately 15–25% of the total protein of the seeds. This fraction is rich in cysteine, methionine and lysine (Boye et al. 2010a, 2010b; Singh 2017; Roy et al. 2010). According to some authors (Boye et al. 2010a, 2010b; Singh 2017), pulse proteins present poor digestibility, which may limit their application to food formulations. The digestibility of the protein varies according to the characteristics of starch present in the seed, which causes it to increase (Singh 2017). Other authors, such as Havemeier et al. (2017), however, report that pulses are considered good sources of digestible proteins. Nosworthy and House (2017), in turn, report that the methods used to determine the protein quality should be considered when assessing it, since the preparation method may alter the quality of the protein.

Carbohydrates correspond to approximately 70% of the composition of pulses. They can be either hydrolyzable by gastrointestinal enzymes, absorbed and metabolized, or those that are unavailable for not being hydrolyzed by digestive enzymes. Starch corresponds to the largest fraction of pulses and is subdivided into amylose and amylopectin. A large variation in the amylose content in pulse starch has been reported. For being rich in complex carbohydrates, pulses have a low glycemic index as they slowly release glucose into the bloodstream. Furthermore, they stimulate prebiotic activity, which is beneficial to health. Bioactive carbohydrates include dietary fibers that include cellulose, hemicellulose, gums, pectins, mucilages, β-glucans, lignins, resistant starch, undigestible oligosaccharides, among others. These substances resist the digestion process and absorption in the small intestine of humans, with partial or complete fermentation in the large intestine. Concerning pulse starch, research demonstrates a strong tendency towards retrogradation and difficulty in swelling and breaking of grains during cooking in comparison to cereal starch. The high fiber content in pulses may increase by satiety (Havemeier et al. 2017; Singh et al. 2017; Tosh and Yada 2010).

Regarding micronutrients, pulses are sources of thiamine, niacin, folate, riboflavin, pyridoxine, potassium, vitamin E, vitamin A, selenium, iron, zinc, calcium and magnesium (Singh 2017; Havemeier et al. 2017; Asif et al. 2013). Recent studies demonstrate that pulses are also rich in polyphenols, tannins, alkaloids, saponins, oxalates, phytates, lectins (hemagglutinins) and enzyme inhibitors (mainly trypsin and chymotrypsin), compounds often considered as antinutritional (Roy et al. 2010). The concentrations of these compounds vary according to the species and variety. Seed coatings, for example, are rich in water-insoluble fibers and polyphenols, while cotyledons are rich in soluble fibers, slow-digesting oligosaccharides and resistant starch (Singh et al. 2017; Mudryj et al. 2014).

Phenolic compounds interfere with protein digestibility in the human organism, leading to low amino acid bioavailability. Tannins, which act as a defense mechanism in plants, have a chelating capacity with metal ions and form hydrogen bonds with proteins, reducing mineral absorption and protein digestibility. They form complexes with starch or its digestive enzymes, as well as conferring astringency sensory characteristics. Phytic acid forms irreversible complexes with proteins and minerals (Roy et al. 2010; Singh et al. 2017).

Despite their antinutritional characteristics, research has suggested that these substances, usually concentrated in seed husks, can prevent several diseases (McCrory et al. 2010; Mudryj et al. 2014). Polyphenols present high therapeutic potential due to their antioxidant activity. Phenolic acids and their derivatives (flavonols, anthocyanins, condensed tannins) are the main categories of polyphenols found in the seeds of leguminous. They act as primary antioxidants by donating hydrogen atoms to free radicals. Its antioxidant action helps prevent the oxidation of lipids, proteins and DNA by species reactive to oxygen which are produced in the cells during oxidation. Ferulic acid is the most abundant phenolic compound followed by p-coumaric acid and sinapic acid. Flavonoid glycosides, anthocyanins and tannins add color to the seed (McCrory et al. 2010; Asif et al. 2013; Mudryj et al. 2014; Singh et al. 2017).

Phytosterols are sterols similar to cholesterol in structure. They have been largely evaluated as functional ingredients for their beneficial health effects, such as reducing cholesterol levels and reducing the intestinal absorption of dietary or endogenous cholesterol. The major phytosterols present in pulses include sitosterol, stigmasterol and campesterol (Havemeier et al. 2017; Singh et al. 2017).

### *7.1.2 Extraction Processes*

Pulses can be processed by using different techniques for producing flour, concentrates and isolates. One of the techniques employed involves spiral airflow classification after grinding, which results in the fractionation of the grains/seeds into high starch flours and high protein flours. The grinding process results in particles that can be separated by size and density. The thinnest fraction corresponds to the protein fraction while the coarser corresponds to the fraction rich in starch (Boye et al. 2010a, 2010b; Sozer et al. 2017).

Alkaline solubilization followed by isoelectric precipitation is a widely used technique for the extraction of proteins from pulses. This technique is based on the solubility of pulse proteins at a higher pH (between 8 and 11) and their low solubility at a pH close to the isoelectric point (4.5). After the isoelectric precipitation, the protein is separated by centrifugation, washed, neutralized and dried. Another technique utilized is the acid extraction of proteins from pulses, which is also based on solubilization, but in an acidic medium (pH lower than 4) with subsequent isoelectric precipitation, cryoprecipitation or membrane separation (Boye et al. 2010a, 2010b).

Pulse proteins can also be extracted in water without further precipitation stages. Successive extractions with a subsequent centrifugation stage are required for a higher yield. Salting-in and salting-out techniques may be employed for protein extraction from pulses. These techniques are based on the ionic strength of saline solutions for the precipitation of proteins, which are subsequently separated by centrifugation (Boye et al. 2010a, 2010b).

Another possibility is the membrane separation, which can be employed as a complement to the isoelectric precipitation. The extracts obtained by acid or alkaline precipitation are concentrated by ultrafiltration processes utilizing membranes selected for the retention of proteins with specific molecular masses (Boye et al. 2010a, 2010b).

### *7.1.3 Functional Properties and Applications*

Due to their nutraceutical and functional properties, pulses are considered promising for composing ingredients in the form of concentrates or isolates of high acceptability and low cost. The industrial application of pulse proteins depends on the physicochemical and structural properties as well as on the conditions of the process (Toews and Wang 2013). Globulins are soluble in saline solutions while albumins are soluble in water. Prolamines and glutenins, which appear in smaller fractions, are soluble in alcohol and basic/acid medium, respectively (Shevkani et al. 2019).

The functional properties of major relevance to the industrial application include the water solubility, emulsifying capacity, water and lipid absorption, foaming and gelling behavior. Such properties vary according to differences in the molecular characteristics of the proteins, such as amino acid composition, molecular mass, secondary structure, charge distribution, hydrophobicity, among others. Albumin has very important characteristics as it interacts and competes with starch due to its solubility in water (Boye et al. 2010a, 2010b; Shevkani et al. 2019).

The solubility is associated with the properties of emulsification, foaming and gelling. From a technological point of view, solubility depends on the balance between hydrophilicity and hydrophobicity of the protein structure. The pH of the medium exerts a strong influence on the solubility of the protein (Boye et al. 2010a, 2010b; Shevkani et al. 2019).

Emulsions consisting of two immiscible liquids in suspension or dispersion form a system of low stability due to interfacial surface tension. To avoid coalescence, it is necessary to promote the stability of the system, which can be achieved by using proteins that form elastic and cohesive layers around the oil droplets. The stability of the emulsion is achieved by the ability of the proteins to increase the viscosity of the continuous phase and to decrease the movement rate of the oil droplets in the system. The emulsifying power has been considered an important property of the pulsed proteins for mass application in meat products, ice cream, soups, mayonnaise among other products (Boye et al. 2010a, 2010b, Shevkani et al. 2019).

Food texture and aroma retention are associated with the capacity of proteins of absorbing lipids and water. Water absorption capacity is important for products such as soups, pasta, creams, bakery products. This capacity is related to the hydrophilic fraction of the protein chains. Fat absorption is important for products such as ground meals, meat substitutes, extenders, baked foods. They are related to the buccal sensation and retention of aromas. These properties are associated with the ability of the nonpolar amino acid fraction to bind to the aliphatic chains of oils and fats. The application of pulses in gluten-free products has been explored due to its viscoelastic capacity in the production of bread and pasta (Boye et al. 2010a, 2010b; Sozer et al. 2017).

Bean curds may also be obtained from pulse proteins, resulting in products similar to tofu, which is soy-based. The foaming capacity is bound to the ability of the proteins to diffuse into the interfaces, reorient themselves and form a viscous film without excessive aggregation. The stability depends on the ability of the protein to maintain the foam. This property is very important for products that require aeration, such as souffles, mousses, ice cream, toppings, among others. The gelling property is important for products such as creams, soups, puddings, meat products among others. This property is obtained when proteins are subjected to heat, pressure and ionic forces (Boye et al. 2010a, 2010b, Shevkani et al. 2019).

Another productive sector of interest for the application of pulses is that of meat products, to obtain texture and yield properties, such as hamburger, sausages, nuggets, and the like (Boye et al. 2010a, 2010b, Shevkani et al. 2019).

Another promising application is the encapsulation for protection of the bioactive and volatile components such as omega 3 fatty acids, phytosterols and carotenoids (Can Karaka et al. 2015). Extruded products such as breakfast cereals, snacks, nut-like products, croutons, crackers and wafer can also be produced (Asif et al. 2013; Sozer et al. 2017).

On the other hand, pulses may give off-taste due to the presence of phenolic compounds and alkaloids. However, this limitation may be corrected or eliminated during processing. In processing, the thermal degradation of the phenolic acids and tamine and the formation of products of Maillard reaction during heating (reaction between amino acids and reducing sugar) may lead to the formation of foreign odors. Oxidation reactions in conjunction with the thermal degradation of carotenoids may also contribute to the formation of foreign odors, as well as degradation of unsaturated fatty acids by the action of lipoxygenases (Roland et al. 2017).

### *7.1.4 Use of Pulses in Dairy Products*

There is little information on the use of pulses as an ingredient for dairy products. Pulse proteins may find applications for products similar to dairy due to their fiber and starch content. However, it is still limited (Asif et al. 2013; Zare et al. 2012).

Cow milk is one of the main raw materials used for producing cheese. However, other sources of milk may be used to produce certain types of cheese, such as the Roquefort which is made from sheep milk. Goat milk is widely used in the production of Chabichou and Sainte-Maure cheese. Buffalo milk, in turn, is widely used for the production of mozzarella cheese.

The *requeijão*, a typically Brazilian product, is classified as fused cheese. It is produced by grinding the mass of any type of cheese (however, depending on the type of cheese utilized, the structure and especially the sensory aspects of the final product may be quite distinct), and then mixing it with some ingredients such as fat, milk powder, whey and emulsifying salts such as polyphosphates. The most prominent product in the Brazilian market is creamy *requeijão* (Cunha et al. 2012; Oliveira et al. 2016). There are different types of *requeijão*, for which the humidity, fat and protein contents vary according to the region and mainly to the manufacturer. The different technologies employed result in products with different rheological characteristics, such as spreadability and texture (Salek et al. 2015; El-Garhi et al. 2018).

The basic composition of a *requeijão* is 58–60% humidity, 1–2% carbohydrate, 1–1.5% sodium chloride, 24–27% fat and 9–11% protein. And presenting 60 to 62% of fat in the dry extract and pH between 5.7 and 6.2. pH has a great influence on the consistency of fused cheeses, especially for *requeijão*, where there is a specific pH range that maintains its past-like form. *Requeijões* with pH from 5.5 to 5.7 have a firm consistency while those with a pH of around 4.9 are drier and those with a pH close 6.5 are softer. (Perry 2004; Van Dender 2014).

The aim in this chapter was to evaluate the potential of pulses in the form of high protein and low starch concentrates as promising ingredients in the partial replacement of animal protein by vegetal proteins in the production of creamy cheese.

## 7.2 Material and Methods

### *7.2.1 Formulations of Requeijões*

In order to establish an initial formulation for this study, a market research was conducted, in which the nutritional composition, based on the label data, of 20 different brands of *requeijão* present in the Brazilian market were evaluated (Table 7.1).

The amount of the ingredients to be used in the formulations of this study was calculated based upon the average composition obtained from the commercial formulations and the requirements of the Brazilian legislation for *requeijão* (Table 7.2).

**Table 7.1** Nutrition facts of some of the leading *requeijões* commonly available in supermarkets

| | Nutrition facts (portion—100 g) | | |
|---|---|---|---|
| Brand[a] | Carbohydrate | Protein | Total fat |
| A | 3.00 | 9.00 | 25.00 |
| B | 0.00 | 7.00 | 27.00 |
| C | 3.33 | 10.33 | 25.00 |
| D | 2.00 | 9.00 | 30.00 |
| E | 4.00 | 10.33 | 21.00 |
| F | 6.00 | 6.66 | 20.66 |
| G | 2.00 | 9.70 | 26.00 |
| H | 4.66 | 4.66 | 21.66 |
| I | 4.66 | 9.33 | 24.00 |
| J | 3.00 | 9.66 | 24.66 |
| K | 2.33 | 10.66 | 21.00 |
| L | 2.66 | 10.00 | 24.00 |
| M | 2.00 | 9.33 | 27.30 |
| N | 0.00 | 10.00 | 23.66 |
| O | 0.00 | 6.67 | 26.67 |
| P | 0.00 | 12.00 | 24.66 |
| Q | 0.00 | 10.00 | 23.00 |
| R | 2.33 | 10.66 | 21.00 |
| S | 4.33 | 10.00 | 30.00 |
| T | 1.66 | 10.66 | 22.66 |
| Average | (2.40 ± 1.79) | (9.28 ± 1.75) | (24.45 ± 2.80) |

Source: Authors, 2019
[a]The brand names are substituted for letters

Finally, from preliminary tests, the replacement of dairy protein by vegetal proteins (pulses) was defined as 25% and 3 pulses (fava bean, pea and lentil) were evaluated, whose proportions varied in each formulation according to levels established by using a simplex factorial mixture design (Table 7.3).

The Geiger equipment, model UMMSK-12E, which consists of a jacketed cooking pan, was used for the production of the *requeijão*. From its total capacity of 12 kg of production, the minimum quantity required for it to function, which is 5 kg, was utilized. The jacketed pan has a homogenizing shovel and cutting knife located inside the bowl. The equipment operates with both direct and indirect steam. To avoid the incorporation of water into the product during manufacture, the indirect steam option was used in this study. The steps adopted for the formulation of the *requeijão* (adapted from Van Dender 2014) are schematically described below.

Cold grinding the mass in Geiger equipment at high rotation for 3 min.

Cold homogenization of the mixture at high rotation (mass, melting salt and NaCl) for 1 min.

Addition of the half part of water and milk cream.

First cooking—heating of the mass under stirring (70–75 °C for 5 min).

**Table 7.2** List of the ingredients utilized in the creamy cheese formulations

| Ingredients | (%) |
|---|---|
| Mineral water | 18–21 |
| Cheese mass | 17–20 |
| Cream | 51–54 |
| Modified corn starch | 1–4 |
| Milk protein concentrate | 2–5 |
| Refined salt | 0.5–1.5 |
| Melting salt | 0.5–1.5 |
| Fava protein | 0–4 |
| Pea protein | 0–4 |
| Lentil protein | 0–4 |
| Potassium Sorbate | 0.10–0.40 |
| Creamy cheese aroma | 0.02–0.10 |
| Lactic acid | 0.10–0.40 |

Source: Authors, 2019

Addition of the remaining ingredients (dairy compound, vegetable protein, potassium sorbate, acids and water).

Second cooking—heating of the mass under stirring (90 °C for 3 min).

Hot jar filling (70–75 °C).

Cooling/Storage (5 °C).

After the production of the formulations, the product was packed in two types of packaging. The part of the product intended for the analysis of viscosity was packed in clear glass jars with a capacity of approximately 210 g, a total height of 103 mm, an outside diameter of 72 mm and a mass of 245 g. The jars were closed with conventional screw-on lids.

The other part of the formulations, intended for the texture and physicochemical analysis, was packed in transparent, low-density polyethylene containers with a total capacity of 200 g, a total height of 92 mm, a diameter of 55 mm at the top margin and 64 mm at the base, with a mass of 3.8 g. An aluminium lid was utilized for this container, which was subsequently fixed by heating, with the aid of manual sealing equipment, Huhtamaki brand, model 111—AD series. Then, a simple snap-on traditional lid was used. Figure 7.1 shows the packaging used for placing the products.

#### 7.2.1.1 Texture Analyses

A Stabela Micro System texturometer TA.XT plus was utilized for the texture analysis. Recently prepared *requeijão* samples (maximum 3 days), which were stored in a refrigerator (8–10 °C), were used. Each sample was analyzed in six repetitions.

**Table 7.3** Factorial mixture design to evaluate the percentage of substitution of dairy protein by vegetal protein (pulses) in the requeijão formulation and its effects on the texture parameters

| Assay | Fava (%) | Pea (%) | Lentil (%) | Firmness (N) | Shear stress (N.s) | Spreadability (N) | Adhesiveness (N) | Viscosity (Pa.s). $10^2$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 25 (1) | 0 (0) | 0 (0) | 9.74 | 6.56 | −13.09 | −2.42 | 7.29 |
| 2 | 0 (0) | 25 (1) | 0 (0) | 7.72 | 5.43 | −10.50 | −1.79 | 6.14 |
| 3 | 0 (0) | 0 (0) | 25 (1) | 8.84 | 6.42 | −11.70 | −2.31 | 7.53 |
| 4 | 12.5 (1/2) | 12.5 (1/2) | 0 (0) | 6.64 | 4.43 | −9.24 | −1.47 | 5.31 |
| 5 | 12.5 (1/2) | 0 (0) | 12.5 (1/2) | 7.90 | 5.56 | −10.56 | −1.84 | 6.85 |
| 6 | 0 (0) | 12.5 (1/2) | 12.5 (1/2) | 8.63 | 6.27 | −11.41 | −2.11 | 7.62 |
| 7 | 16.6 (2/3) | 4.2 (1/6) | 4.2 (1/6) | 7.78 | 5.46 | −10.50 | −1.71 | 6.76 |
| 8 | 4.2 (1/6) | 16.6 (2/3) | 4.2 (1/6) | 7.88 | 5.62 | −10.64 | −1.77 | 6.15 |
| 9 | 4.2 (1/6) | 4.2 (1/6) | 16.6 (2/3) | 7.57 | 5.28 | −10.34 | −1.73 | 6.45 |
| 10 | 8.3 (1/3) | 8.3 (1/3) | 8.3 (1/3) | 7.37 | 5.02 | −10.20 | −1.64 | 6.42 |
| 11 | 8.3 (1/3) | 8.3 (1/3) | 8.3 (1/3) | 7.75 | 5.29 | −10.71 | −1.75 | 6.75 |
| 12 | 8.3 (1/3) | 8.3 (1/3) | 8.3 (1/3) | 8.47 | 5.83 | −10.88 | −1.95 | 6.93 |
| Control[a] | – | – | – | 5.75 | 3.28 | −8.77 | −1.25 | 4.32 |

Source: Authors, 2019

[a]Control = *requeijão* without the addition of pulses

All analyses were performed in six replicates and the coefficient of variation (CV) ranged: firmness: from 1.5 to 7.2%; shear stress: from 2.9 to 8.6%; spreadability: from 1.7 to 6.1%; adhesiveness: from 5.4 to 16.9; viscosity: from 0.4 to 14.7%

For each analysis, approximately 10 g of the sample, with a temperature previously set to 20 ° C, was placed in a female cone and pressed to eliminate air pockets. The excess sample was removed with a knife until the surface was flat.

After the preparation of the sample, the female cone was placed in the texturometer holder and fitted to the male cone. The two cones were aligned and the measurement started by penetrating the probe contained in the male cone into the sample. The probe penetrated the sample by 23 mm and then returned to the surface. The force required for the probe to reach the maximum depth (23 mm) was defined as the firmness of the sample; the application area of the penetration force was defined as shear stress. When the probe was removed from the sample, the force applied defined the spreadability and the negative area of the application of force, the adhesiveness. Figure 7.2 illustrates the functioning of the texturometer for the

**Fig. 7.1** Picture of creamy cheese packages. Source: Authors, 2019

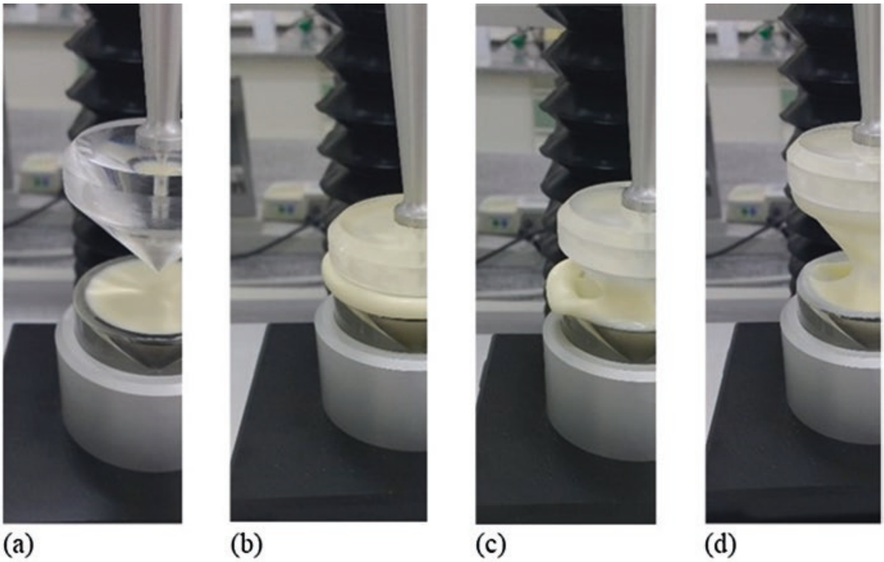


**Fig. 7.2** Texture analysis steps of *requeijão* samples, being (**a**) and (**b**) probe penetration steps to measure firmness and shear stress and (**c**) and (**d**) probe removal to measure spreadability and adhesiveness . Source: Authors, 2019

measurements performed. The procedure followed the instructions provided by the texturometer manufacturer.

The analysis of viscosity was conducted with samples stored in glass flasks three days after processing. A Brookfield viscometer model RVDV-II + Pro containing a

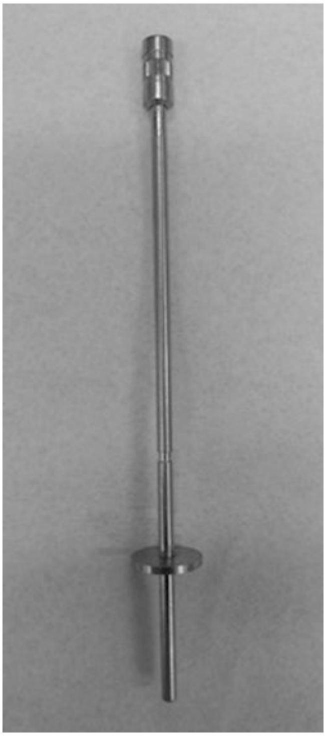

**Fig. 7.3** Spindle number 6 applied to measure viscosity of *requeijão* samples. Source: Authors, 2019

spindle of number 6 (as shown in Fig. 7.3) was used. 10 rpm rotation speed and 50% torque were applied. The temperature of the samples was maintained between 10 °C and 12 °C during the analysis.

## 7.3 Physicochemical Analyses

The physicochemical analyses applied to the *requeijão* formulation were: pH, humidity, color (L, *a*, *b*), protein and lipids. The pH was measured by direct reading of the pH of the samples at room temperature using bench pH meter. The analysis of humidity analysis was performed utilizing the oven method, drying the samples at 105 °C, at atmospheric pressure, until the samples achieved constant weight. The color (L, *a*, *b*) was measured utilizing a spectrophotometer (Konical Minolta, model CM-2600D). The protein analysis was performed by acid digestion of samples followed by reaction with concentrated sodium hydroxide, sample distillation, collection of nitrogen fraction in boric acid solution and titration with hydrochloric acid, according to Kjeldahl method. Lipid analysis was performed by acid digestion of the sample in the presence of isoamyl alcohol, according to Gerber method (Zenebon and Pascuet 2008).

## 7.4 Results and Discussion

From the data displayed in Table 7.1, the *requeijão* cheese formulation was defined for this study containing 9–10% protein, 24–26% lipids and 3–5% carbohydrates. These macronutrient concentration ranges were used to calculate the concentration ranges of each ingredient to be added in the formulations as shown in Table 7.2.

Table 7.3 presents the quantities of each type of pulse used for each formulation of *requeijão*. The percentages indicated refer to the substitution made concerning the milk protein. In all assays, the substitution was 25% of milk protein for vegetable protein, whose ratio/type of vegetable protein is variable in each test. The answers obtained for the parameters of texture are also presented in Table 7.3. In addition to the texture analyses, physicochemical analyses of the *requeijão* formulations were performed, whose results can be seen in Table 7.4.

**Table 7.4** Factorial mixture design to evaluate the percentage of substitution of dairy protein by vegetal protein in the *requeijão* formulation and its effects on the physicochemical parameters

| Assay | Fava (%) | Pea (%) | Lentil (%) | pH | Humidity (% w/w) | Protein (%w/w) | Lipid (% w/w) | L | a | b |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 25 (1) | 0 (0) | 0 (0) | 5.93 | 59.06 | 9.06 | 25.35 | 84.41 | −0.71 | 19.16 |
| 2 | 0 (0) | 25 (1) | 0 (0) | 5.84 | 59.29 | 9.39 | 25.52 | 84.50 | 0.71 | 22.10 |
| 3 | 0 (0) | 0 (0) | 25 (1) | 5.84 | 58.95 | 9.41 | 22.95 | 84.45 | 0.17 | 19.65 |
| 4 | 12.5 (1/2) | 12.5 (1/2) | 0 (0) | 5.84 | 59.33 | 9.74 | 25.87 | 84.36 | 0.05 | 20.36 |
| 5 | 12.5 (1/2) | 0 (0) | 12.5 (1/2) | 5.86 | 59.43 | 9.19 | 24.79 | 84.47 | −0.21 | 19.03 |
| 6 | 0 (0) | 12.5 (1/2) | 12.5 (1/2) | 5.83 | 58.82 | 9.90 | 26.06 | 86.10 | 0.51 | 20.41 |
| 7 | 16.6 (2/3) | 4.2 (1/6) | 4.2 (1/6) | 5.85 | 59.35 | 9.53 | 26.56 | 83.92 | −0.30 | 18.75 |
| 8 | 4.2 (1/6) | 16.6 (2/3) | 4.2 (1/6) | 5.77 | 59.18 | 9.28 | 25.04 | 84.18 | 0.33 | 20.39 |
| 9 | 4.2 (1/6) | 4.2 (1/6) | 16.6 (2/3) | 5.80 | 59.35 | 9.56 | 25.19 | 83.07 | 0.04 | 19.04 |
| 10 | 8.3 (1/3) | 8.3 (1/3) | 8.3 (1/3) | 5.82 | 59.44 | 9.65 | 24.02 | 84.00 | 0.05 | 19.57 |
| 11 | 8.3 (1/3) | 8.3 (1/3) | 8.3 (1/3) | 5.86 | 59.38 | 9.30 | 22.67 | 84.77 | 0.13 | 19.34 |
| 12 | 8.3 (1/3) | 8.3 (1/3) | 8.3 (1/3) | 5.84 | 59.29 | 9.47 | 26.00 | 84.46 | 0.09 | 19.37 |
| Control[a] | – | – | – | 5.93 | 59.77 | 9.80 | 22.18 | 85.48 | −0.94 | 15.59 |

Source: Authors, 2019

[a]Control = *requeijão* without the addition of pulses

All analyses were performed in three replicates and the coefficient of variation (CV) ranged: pH: from 0.1 to 0.4%; humidity: from 0.02 to 0.76%; protein: from 0.2 to 6.8%; lipid: from 0.4 to 6.7; L: from 0.4 to 14.7%; a: from 6.1 to 269.5% and b: from 1.0 to 2.2%

Color *a* presented high values of CV in samples that average were very close to zero

For each texture (Table 7.3) and physicochemical (Table 7.4) parameter, linear mathematical models (with and without interactions) were evaluated to explain the variation observed of the properties of the *requeijão*. The results can be seen in Table 7.5. A response surface analysis (Fig. 7.3) was also performed for each model that was considered statistically significant and predictive. The models with high reliability ($p < 0.05$) and high correlation (correlation coefficient greater than 0.85) were considered.

All texture responses presented an increase concerning the control formulation which contained dairy protein only (the increases compared to the control were: from 15.5 to 74.9% for firmness, from 34.6 to 99.7% for shear stress, from 5.3 to 49.2% for spreadability and from 18.1 to 93.7% for adhesiveness). However, the effect of each pulse was different in each response evaluated. A greater increase in the value of the variables firmness and shear stress responses was observed as a function of the increase in the concentrations of fava bean and lentil, whereas the replacement by pea caused a less significant increase in these parameters. There was a negative interaction between fava bean x pea and fava bean x lentil, which indicates that pulse mixture formulations were less firm and with have less shear stress than those that used pulses in isolation.

Regarding the spreadability and adhesiveness, as the parameters present a negative sign, the analysis was made according to the modulus of these responses. Thus, the results observed presented the same tendency of the first two variables, that is,

**Table 7.5** Mathematical models for texture and physicochemical parameters for *requeijão* with partial replacement of milk protein by vegetal proteins (fava beans, peas and lentils)

| Parameter | Linear model equation[a] | *p*-value | $R^2$ | $R^2$ adjusted | R |
|---|---|---|---|---|---|
| **Firmness** | **FI = 9.62 F +8.02 P +8.84 L −6.90 (F.P) −4.71 (F.L)** | 0.03 | 0.75 | 0.60 | 0.87 |
| **Shear stress** | **SH = 6.54 F +5.69 P +6.40 L −5.39 (F.P) −3.43 (F.L)** | 0.03 | 0.75 | 0.60 | 0.87 |
| **Spreadability** | **SP = −12.91 F −10.83 P −11.73 L +8.40 (F. P) +6.15 (F.L)** | <0.01 | 0.83 | 0.73 | 0.91 |
| **Adhesiveness** | **ST = −2.39 F −1.85 P −2.28 L +2.28 (F.P) +2.02 (F.L)** | <0.01 | 0.84 | 0.74 | 0.92 |
| **Viscosity** | **V = 7.19 F +6.14 P +7.12 L −5.03 (F.P) +3.09 (P.L)** | 0.02 | 0.77 | 0.64 | 0.88 |
| pH | pH = 5.90 F +5.80 P +5.82 L | 0.04 | 0.50 | 0.39 | 0.71 |
| **Humidity** | **H = 59.03 F +59.25 P +58.98 L +0.89 (F.P) +2.11 (F.L) −0.81 (P.L)** | 0.02 | 0.85 | 0.72 | 0.92 |
| Protein | P = 9.23 F +9.61 P +9.53 L | 0.39 | 0.19 | 0.01 | 0.44 |
| Lipid | LP = 25.07 F +23.57 P +25.56 L | 0.51 | 0.14 | 0.00 | 0.37 |
| *L* | *L* = 84.05 F +84.74 P +84.38 L | 0.72 | 0.07 | 0.00 | 0.26 |
| **Color *a*** | *a* = **−0.68 F +0.72 P +0.18 L** | <0.01 | 0.99 | 0.98 | 0.99 |
| **Color *b*** | *b* = **18.95 F +21.59 P +19.40 L −3.05 (F.L)** | <0.01 | 0.87 | 0.82 | 0.93 |

[a]In all equations fava beans, peas and lentils are represented by letters F, P and L, respectively
Models marked in bold are considered statistically significant and predictive

the increase of the fava bean and lentil concentrations promoted a greater increase of spreadability and adhesiveness and there was a negative interaction (decrease of these parameters) in the formulations with the pulse combinations fava beans x peas and fava beans x lentils.

The viscosity, in turn, presented a different behavior, and the highest values for this parameter were obtained by the formulations with higher lentil concentration and the interactions of the variables occurred in the opposite direction. While there was a negative interaction (viscosity reduction) between fava bean x pea, a positive interaction (increased viscosity) between lentil x pea was observed.

The two main findings of the present study were that: (1) the partial substitution of dairy protein by vegetal protein caused an increase in the values of all the parameters related to texture evaluated, that is, all texture parameters were higher in the formulations containing pulses than in the control condition that used milk protein only (Table 7.3); (2) the use of fava bean and lentil proved to increase texture parameters more than the use of pea.

The functional properties of the *requeijão* depend on several factors such as the characteristics of the milk proteins, fat and melting salts content, processing conditions as well as melting temperature and the pH of the final product. However, in this study, all these conditions were maintained constant and the only variations between formulations concern the type and concentration of the pulses utilized. Therefore, the increases in the texture parameters must be related to the use of vegetal proteins.

According to Boye et al. (2010a, 2010b), the amino acids that appear most in pulses are glutamine, aspartic acid, arginine, lysine, methionine and cysteine, which are all polar amino acids (except for methionine). On the other hand, Fox et al. (1998) state that the main amino acids present in caseins are valine, leucine, isoleucine, phenylalanine, tryptophan and proline, which are nonpolar amino acids. In this way, the partial replacement of dairy protein by proteins from pulses promotes an increase in the content of polar amino acid and, consequently, an increase in the hydrophilic character of the proteins present in the *requeijão*, increasing the protein-water interaction, which helps to explain the increase in the texture parameters of the product.

The differences in the effects of each pulse may also be related to the specific composition of each of them, the pulse-pulse and pulse-casein interaction, and the formation of protein co-aggregates (pulse-pulse and pulse-casein) that were three-dimensional networks of variable stability. All these factors must be better studied for a complete understanding of the action of pulses as ingredients in dairy products such as *requeijão*.

There are few studies in the literature comparing the different types of pulse, especially in dairy products. Fernández-Quintela et al. (1997) reported similarity in the capacity of water absorption of fava bean and pea pulses. However, this author verified that the fava pulse had a greater capacity of fat absorption than the pea pulse. Such observation may help explain the lower values of texture parameters in formulations with higher pea concentration. The lower interaction of the pea pulse with fat leaves this component freer in the medium and it is well known that fat is a factor that promotes the reduction of viscosity in *requeijão* (Van Dender 2014). A similarity between the emulsifying power of fava, pea and lentil pulses was also observed in other studies (Boye et al. 2010a, 2010b; Shevkani et al. 2019). Such similarity may support the hypothesis that the differences between products with and without the addition of pulses must be related to the interaction of the pulses with milk protein and the other ingredients of the formulation.

The statistical evaluation for pH indicated that the interactions between pulses were not significant and that although the model (Table 7.5) presented p-value less than 0.05, the determination and correlation coefficients were very low. Therefore, the model obtained was not considered statistically significant and predictive. The pH values were all very similar (5.77–5.93), which can be explained by the use of lactic acid as an acidity regulator. The values obtained in this study agree with the pH value range in commercial *requeijão* sold in the Brazilian market, which generally ranges from 5.7 to 6.0 (Van Dender 2014).

The humidity analysis also indicated very similar values between the different formulations. However, there was considerable positive interaction between the fava bean x lentil which increased the humidity by two percentage points on average. The statistical analysis indicates that there was a synergy between these two pulses in water retention in the product, which may help explain why there was a negative interaction between these variables in the texture parameters. A greater quantity of water in the final product is a factor that can lead to decreased firmness, shear stress, spreadability and adhesiveness.

According to da Silva et al. (2012), the increase in the humidity content in light cheese is a factor that compensates for the reduction in fat content, since it promotes the dissolution of the protein matrix, compensating for the increase in viscosity caused by fat reduction. Similarly, in this study, the addition of pulses caused an increase in the texture parameters, thus, the higher final humidity content may exert the same effect verified in light cheeses, that is, to promote the dissolution of the protein matrix reducing the values of the texture parameters.

Due to the standardization of ingredients adopted in this study, the protein and lipid contents of all formulations were very similar and, therefore, no statistically significant models were obtained for these physicochemical parameters.

Regarding color, the parameter L (luminosity) did not present a statistically significant model. Table 7.4 indicates that the results were practically the same for all formulations. For the parameter a (red/green coordinate), however, large differences were observed in the use of each pulse: increasing the concentration of the fava pulse increased the green color whereas increasing the concentration of the pea pulse increased the color red. This difference must be assessed carefully from an industrial point of view as it can have a major influence on the sensory acceptance of consumers. Comparing the color of the formulations with the control, the control was the greenest of all ($a = -0.94$) and the addition of the pulses altered the color of the product increasing the red color, being the fava bean pulse the least influencing for this response and the pea pulse was the one that changed the color of the formulations the most.

As for parameter b (yellow/blue coordinate), the coefficients of all pulses were positive and higher than the control formulation, indicating that pulse use intensified the yellow coloration of the product. The formulations with pea pulse presented the highest intensity of the yellow color by the response surface analysis. However, there was a negative interaction between the fava bean and pea, that is, the association of these two pulses left the color yellow less bright, which is also an issue to be observed from the sensory point of view.

There is a tendency of *requeijão* formulations to have a whiter and less yellow coloration in the Brazilian market generally. The increase in yellow color may be a negative factor for the sensory acceptance of the product. In the present study, the average increase of the yellow color of *requeijão* produced utilizing pulses was 26.8% compared with the control formulation. This difference may have an impact on the sensorial acceptance of *requeijões* contained pulses. Cunha et al. (2010) evaluated the production of *requeijão* with substitution of dairy fat by hydrogenated vegetal fat (25 and 50% substitution) and found that the formulation with 50% fat substitution was the one with the lowest yellow coloration (parameter b) and was also the one with the highest sensory acceptance of color in tests with consumers.

## 7.5 Conclusion

The study presented in this chapter represents a contribution to the literature on the application of pulses in dairy products. The data obtained demonstrated that the substitution of 25% of milk protein for fava bean, pea and/or lentil pulses is possible for the production of *requeijão* (a typical Brazilian cream cheese) with characteristics similar to those of the product obtained with milk protein only, in the process conditions utilized.

The partial substitution of milk protein for pulses had a greater impact on the texture, which increased all texture parameters analyzed (firmness, shear stress, spreadability, adhesiveness and viscosity). The most probable causes for these alterations are the increased amount of polar amino acids (which affects the interaction of the protein matrix with the water and fat of the product), the formation of protein co-aggregates, and pulse-pulse, pulse-casein interactions and pulse interactions with other ingredients of the formulation. The processing conditions such as pH, ionic strength, temperature and agitation also contribute to denaturation and alteration of the proteins, influencing the final results.

The type of pulse utilized has an impact on the variation of the final properties of the product. The fava bean pulse caused the greatest alterations in texture whereas the pea pulse changed the color of the formulations significantly compared to the control formulation. On the other hand, the use of mixtures of pulses presented few interactions, being the most relevant those related to the final humidity of the product, in which the interaction of the pulses of fava bean and lentil increased while the interaction between the pulses of pea and lentil decreased it. The use of the pulses had an impact on the final cost of the product, with a reduction of cost depending on the concentration and type of pulse utilized, which is promising in the food industry. Finally, the advancement in studies of substitution of animal raw materials for vegetal ones may promote impacts regarding sustainability in food production and nutritional issues for consumers, which are relevant subjects for further research.

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## References

Asif M, Rooney LW, Ali R, Riaz MN (2013) Application and opportunities of pulses in food system: a review. Crit Rev Food Sci Nutr 53:1168–1179

Boye JI, Aksay S, Roufik S, Ribéreau S, Mondor M, Farnworth E, Rajamohamed SH (2010a) Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Food Res Int 43:537–546

Boye J, Zare F, Pletch A (2010b) Pulse proteins: processing, characterization, functional properties and applications in food and feed. Food Res Int 43:414–431

Can Karaka A, Low NH, Nickerson MT (2015) Potential use of plant proteins in the microencapsulation of lipophilic materials in foods. Trends Food Sci Technol 42:5–12

Cunha CR, Dias AI, Viotto WH (2010) Microstructure, texture, color and sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. Food Res Int 43:723–729

Cunha CR, Alcântara MR, Viotto WH (2012) Effect of the type of emulsifying salt on microstructure and rheological properties of "Requeijão Cremoso" processed cheese spread. J Food Sci 77:E176–E181

da Silva RDCSN, Minim VPR, de Lima LP, Gomide AI, da Silva Moraes LE, Minim LA (2012) Otimização da aceitabilidade sensorial de requeijão cremoso light. Cienc Rural 42:360–366

El-Garhi H-EM, El-Aidie SA, Rashid NA, Hayee ZA (2018) Quality improvement of spreadable processed cheese made from ultrafiltered milk retentates using commercial starter cultures. Food Sci. Technol Int 24:465–475

Fernández-Quintela A, Macarulla MT, Del Barrio AS, Martínez JA (1997) Composition and functional properties of protein isolates obtained from comercial legumes grown in northem Spain. Plant Foods Hum Nutr 51:331–341

Food and Agricultural Organization of the United Nations (2016) What are pulses? http://www.fao.org/pulses-2016/news/news-detail/en/c/337107/. Accessed 2 Dec 2019

Fox PF, Uniacke-Lowe PLH, McSweeney PLH, O'Mahony JA (1998) Milk proteins. In: Fox PF, Uniacke-Lowe PLH, McSweeney PLH, O'Mahony JA (eds) Dairy chemistry and biochemistry. Springer, Cham, pp 145–239

Havemeier S, Erickson J, Slavin J (2017) Dietary guidance for pulses: the challenge and opportunity to be part of both the vegetable and protein food groups. Ann N Y Acad Sci 1392:58–66

Mccrory MA, Hamaker BR, Lovejoy JC, Eichelsdoerfer PE (2010) Pulse consumption, satiety, and weight management. Adv Nutr 1:17–30

Mudryj AN, Yu N, Aukema HM (2014) Nutritional and health benefits of pulses. Appl Physiol Nutr Metab 39:1197–1204

Nosworthy MG, House JD (2017) Factors influencing the quality of dietary proteins: implications for pulses. Cereal Chem 94:49–57
Oliveira RBA, Margalho LP, Nascimento JS, Costa LEO, Portela JB, Cruz AG, Sant'anna AS (2016) Processed cheese contamination by spore-forming bacteria: a review of sources, routes, fate during processing and control. Trends Food Sci Technol 57A:11–19
Perry KSP (2004) Queijos: Aspectos químicos, bioquímicos e microbiológicos. Quim Nova 27:293–300
Roland WSU, Pouvreau L, Curran J, Van De Velde F, De Kok PMT (2017) Flavor aspects of pulse ingredients. Cereal Chem 94:58–65
Roy F, Boye JI, Simpson BK (2010) Bioactive proteins and peptides in pulse crops: pea, chickpea and lentil. Food Res Int 43:432–442
Salek RN, Cerníkova M, Nagyová G, Kuchar D, Bacová H, Minarciková L, Bunka F (2015) The effect of composition of ternary mixtures containing phosphate and citrate emulsifying salts on selected textural properties of spreadable processed cheese. Int Dairy J 44:37–43
Shevkani K, Singh N, Chen Y, Kaur A, Yu L (2019) Pulse proteins: secondary structure, functionality and applications. J Food Sci Technol 56:2787–2798
Singh N (2017) Pulses: an overview. J Food Sci Technol 54:853–857
Singh B, Singh JP, Shevkani K, Singh N (2017) Bioactive constituents in pulses and their health benefits. J Food Sci Technol 54:858–870
Sozer N, Holopainen-Mantila U, Poutanen P (2017) Traditional and new food uses of pulses. Cereal Chem 94:66–73
Toews R, Wang N (2013) Physicochemical and functional properties of protein concentrates from pulses. Food Res Int 52:445–451
Tosh SM, Yada S (2010) Dietary fibers in pulse seeds and fractions: characterization, functional attributes, and applications. Food Res Int 43:450–460
Van Dender AGF (2014) Requeijão cremoso e outros queijos fundidos: Tecnologia de fabricação, controle do processo e aspectos de mercado, Metha, São Paulo, Brasil
Zare F, Champagne CP, Simpson BK, Orsat V, Boye JI (2012) Effect of the addition of pulse ingredients on acid production by probiotic and yoghurt starter cultures. LWT Food Sci Technol 45:155–160
Zenebon, O., Pascuet, N.S., and Tigela, P. (org)., 2008, Métodos físico-químicos para análise de alimentos, Instituto Adolfo Lutz, São Paulo