Physicochemical and rheological properties of lentil milk/inulin blend—A feta cheese analogue

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Abstract
In recent years, due to nutritional conditions and increasing consumer awareness, the tendency of using healthy food has been necessary. Besides, much consideration is given to use of the legumes protein and fat in dairy products due to their low cost and healthy benefits. Indeed, by increasing obesity and cardio-vascular diseases, and consideration of consumers to nutritional health, the demand for healthy products has increased. The present study is based on achieving a good formulation by replacing a part of cow's milk with lentil milk to produce vegetable functional cheese with good flavor and texture and acceptable appearance from the point of view of consumers. Inulin was used to improve the texture and organoleptic properties. The applied lentil milk was 0%, 10%, and 20%, and inulin was 0%, 3.5%, and 7.0% in nine formulations. The results revealed that the high level of lentil milk (20%) lead to a sandy texture. The formulation which contained 10% and 3.5% of lentil milk and inulin, respectively, obtained the best texture and sensory score.

Keywords
analogue cheese, functional dairy products, inulin, lentil milk

1 | INTRODUCTION

Although dairy products are rich in nutrients such as calcium, protein, potassium, and phosphorus (Walther et al., 2008) but several studies confirmed the direct relationship between dairy consumption and increased risk of chronic diseases like type 2 diabetes, obesity, cardio-vascular disease, cancer, and osteoporosis, indeed, natural cheese has a higher cost of production and storage. Therefore, the replacement of milk fat with free or low-fat natural sources could decrease the serum cholesterol level in humans (Thorning et al., 2016). Recently, food scientists are interested in analogue cheeses because of their lower costs and easy manufacturing (Bachmann, 2001). Analogue cheese or imitation cheese is a kind of synthetic cheese, which uses fat and/or protein from other sources in their manufacturing process; it means that a part of milk was replaced by plant sources. Also, the importance of legume foods in the promotion of healthy and functional foods is increasing worldwide. Several studies have proved the beneficial health uses of legumes due to their macro- and micro-nutritional compounds and bioactive phytochemicals. Lentil (Lens culinaris L.) is a member of the Leguminoceae family and one of the ancient and the most commonly used legume which is cultivated all around the world (Faris et al., 2013; Salman & Hasan, 2014). Lentil seeds are the major source of proteins, vitamins (B and folate), minerals (potassium, calcium, phosphor, magnesium, iron, cobalt, and zinc), and complex carbohydrate (dietary fiber, starch, and oligosaccharides); the fat level in lentil is less than 1% (Kowieska & Petkov, 2003; Wang & Toews, 2011); and the level of protein is 20.6–31.4% (Urbano et al., 2007). Joshi et al. (2017) asserted that the level of protein in lentil seeds and meat is almost the same and is twice as much as the level of protein in most of the other legumes. The high level of protein in lentil seed makes it appropriate for functional human foods (Khazaei et al., 2019). Zare (2011) used lentil flour...
instead of skim milk in preparing probiotic yogurt; their results showed that 1% to 3% of lentil flour causes a decrease in pH and enhances the fermentation because of the low buffer capacity of lentil flour. A salad dressing incorporated with raw and thermally treated lentil flour showed the shear-thinning behavior; also, heat treatment on lentil seeds caused a decreasing in the non-Newtonian fluid of the samples (Ma et al., 2013). Lentil flour in stirred yogurt increased the consistency and thixotropic index of samples (Küçükçetin et al., 2012). Also, several studies confirmed the effectiveness of lentils in cracker snack (Han et al., 2010; Ryland et al., 2010), bakery field (Aider et al., 2012), layer, and sponge cake (De la Hera et al., 2012). The present study evaluates the feasibility of producing vegetable cheese by replacing a part of cow’s milk with lentil milk. Inulin was used to improve tissue properties. Inulin is a type of fructans, which is colorless and odorless but improves organoleptic characteristic because of its slightly sweet taste (Nair et al., 2010).

2 MATERIAL AND METHOD

2.1 Materials and reagents

Lentil was purchased from a local store in Tehran, Iran. Milk was prepared from a local dairy product. Other ingredients used in this study were acetic acid (distilled vinegar from Amol Etminal company with concentration 5%) and inulin (SENSUS brand, 99.5%).

2.2 Lentil milk

Raw lentil seeds were cleaned and washed and then soaked in water for 14 h. The husk of the seeds was removed manually and then mixed with water for 3 min by kitchen blender (Moulinex, France) to obtain a homogenous mixture. The mixture filtered through a cheesecloth. First, the amount of dry matter in both cow’s milk and lentil milk was measured, which was 13% and 18.33%, respectively. The dry matter in lentil milk and inulin was assimilated to raw milk using Equation 1. For this purpose, 13.83 g of inulin and 70.92 g of lentil milk were blended with 86.17 and 29.08 g of water separately, to obtain 100 g of inulin and lentil milk with 13% dry matter.

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\frac{\text{Dry matter of solution}}{\text{Dry matter}} \times 100
\]

2.3 Cheese analogue making

The cheese samples were made of three levels of lentil milk (0%, 10%, and 20%) and three levels of inulin (0%, 3.5%, and 7.0%) in nine formulations according to Table 1.

At the first step, raw milk, lentil milk, and inulin were weighted at the mentioned ratios in Table 1 by a digital scale and were poured into the boiler with 10 kg capacity. Then, it was heated to its boiling point. During the heating, the mixture was constantly stirred to the same heat distribution. As soon as the mixture reached boiling point, the heating stopped, 125 g acetic acid was added to the mixture, and stirring was continued. After 15 min, the mixture was passed through cheesecloth and pressed by resting 2 kg weights on the curd; 10 h later, the weights were doubled (4 kg) to boost the drainage of whey. After 6 h of pressing and drainage, the cheese was placed in the 7% saltwater and stored in the refrigerator for a week until further analysis. All analysis was done in triplicate.

2.4 Solid matter content

The dry matter of samples was determined according to standard No. 1753 (ISIRI, 2006a). Briefly, the weight of samples was measured before and after drying in the oven at 102 ± 2°C.

2.5 Determination of pH and acidity

2.5.1 The pH value was recorded using a pH-meter (pH-meter PL-700PV, GONDO, Taiwan)

Briefly, 10 g of cheese sample was homogenized with 50 ml distilled water, and the pH of the dispersion was measured according to standard No. 2852, (ISIRI, 2007).

| TABLE 1 Cheese samples formulations |
|------------------------------------|
| Sample | Raw milk (%) | Inulin (%) | Lentil milk (%) | Formula |
| Control object | 100 | 0 | 0 | L1I1 |
| 1 | 86.5 | 3.5 | 10 | L1I2 |
| 2 | 83 | 7 | 10 | L1I3 |
| 3 | 76.5 | 3.5 | 20 | L2I2 |
| 4 | 96.5 | 3.5 | 0 | L2I3 |
| 5 | 93 | 7 | 0 | L3I2 |
| 6 | 76.5 | 3.5 | 20 | L3I3 |
| 7 | 90 | 0 | 10 | L4I1 |
| 8 | 73 | 7 | 20 | L4I3 |
2.5.2 | Acidity was determined by endpoint titration using 0.1 N NaOH

Twenty grams of homogenized sample added to 250 ml volumetric flask and diluted with distilled water and then passed through Whatman No. 42 filter paper. Twenty-five-milligram portions of the filtrate were titrated with NaOH against indicator (0.1% Phenolphthalein) (standard No. 2852, ISIRI, 2007).

2.6 | Protein content

The crude protein content in milk and cheese was determined by measuring total nitrogen using Kjeldahl method (standard NO. 639, 9188; ISIRI, 2006b, 2015). All kjeldahl determinations were done using Kjeltec Auto Analyzer (Hoganas, Sweden). Lentil milk was analyzed for protein using the method of Association of Official Analytical Chemists (AOAC, 2012).

2.7 | Texture profile analysis

Texture analysis of the cheese samples was performed according to a method described by Gunasekaran and Mehmet (2003). Cheese samples were cut into cubes (20 mm × 20 mm × 20 mm). TPA of samples was carried out using a texture analyzer (CT3, Brookfield), and a double-bite compression cycle was carried out with a rest period of 10 s. Optimal test conditions were at 50% compression, 36 mm diameter cylinder probe, and force speed 60 mm/min. The firmness, cohesiveness and gumminess was calculated according to Figure 1.

2.8 | Sensory evaluation

Sensory analysis was performed using a 5-point Hedonic scale method (Hossini et al., 2013). Sensory quality was assessed by 30 trained panelists (15 females and 15 males). Ten minutes before the analysis, the cheese samples were taken out of the refrigerator, and the experiments were performed at room temperature. After testing the samples, panelists filled out the prepared forms. The forms consisted of three evaluation criteria: taste, stiffness, and spreadability, as the stiffness was evaluated by the force required to cut the samples with a knife by panelists. The spreadability degree of the samples was measured by evaluating the spreading rate of samples by a knife on toasted bread. Then, panelists scored the samples according to their experience and interest from 1 to 5 (1: terrible; 5: fantastic).

2.9 | Statistical analysis

In this study, lentil milk and inulin were used to produce vegetable cheese with nine different formulations based on a completely randomized factorial design. Analysis of variance (ANOVA) was performed using Minitab 16.1. Data analysis and creating charts were done by Minitab and Excel. All experiments were performed in triplicate.

3 | RESULTS

3.1 | Evaluation of chemical analysis

The ANOVA results for the effect of factors (inulin, lentil milk, and the interaction effect between inulin and lentil milk) on chemical properties are tabulated in Table 2. ANOVA showed that the effect of inulin on pH was not significant, but lentil milk had a minor effect on the pH; the interaction effect between inulin and lentil milk on the pH was significant (p < 0.001).

The comparison of the mean results of the effect of factors on the pH showed that at the zero and low levels of lentil milk (10%), the addition of inulin had no considerable effect on pH (Figure 2a), which were in harmony with the results found by Guggisberg et al. (2009).
They showed that adding inulin to yogurt did not have a considerable effect on the pH value.

The effect of inulin on acidity was not significant, but the effect of lentil milk and the interaction effect between inulin and lentil milk on the acidity was significant (p < 0.001). At zero and 10% of lentil milk, the acidity did not change by increasing the level of inulin. The high level of lentil milk reduced the pH; also, adding inulin to the samples with 20% lentil milk caused a slight decrease in pH, which means increased acidity in these samples (Figure 2b). The acidity was absolutely correlated to the pH value because as the pH decreased, the acidity increased.

The effect of all three factors (inulin, lentil milk, and their interaction effect) on the solid matter was significant (p < 0.001). According to Table 3, the amount of solid matter in the samples containing inulin without lentil milk was similar to the control sample. The addition of lentil milk reduced the solid matter of the samples, which samples containing 20% lentil milk had the lowest amount of solid matter compared to other samples.

The protein content in milk, control cheese (cheese without lentil milk and inulin), and lentil milk was 4.37, 18.23, and 27.04 percentage, respectively. Table 3 shows the protein content in cheese samples supplemented with different levels of lentil milk and inulin. The effect of lentil milk and inulin on protein content was highly significant (p < 0.01). The highest protein content was related to the samples containing the third level of both lentil milk and inulin (L3I3); also, other samples had more protein content compared to the control sample.

### 3.2 Sensory analysis

The ANOVA results in Table 4 show the effect of inulin and lentil milk on the sensory parameters (taste, stiffness, spreadability, and total acceptance). According to ANOVA, the effect of inulin on the taste was not significant, but the effect of lentil milk and the interaction effect between lentil milk and inulin were significant. Lentil milk had considerable effect on the all parameters.

Stiffness is defined as the force required to cut the cheese with a knife. ANOVA showed that the effect of inulin and the interaction effect between lentil milk and inulin on the stiffness was not significant but the effect of lentil milk on stiffness was considerable. According to Figure 3a, the addition of lentil milk reduced the stiffness. Cheese samples supplemented with 20% lentil milk had a sandy texture so did not have a proper texture for texture analysis.

The effect of inulin on spreadability was not significant, but lentil milk had a considerable effect (0.01 > P > 0.001); also, the interaction effect between lentil milk and inulin was significant (P < 0.001). There was no significant difference between the supplemented and control samples in spreadability (Figure 3b).

Based on Figure 3c, the control sample gave the highest taste score among all samples and also had the highest acceptance rate by panelists. The formulations which contain inulin without lentil milk did not affect the taste; as already observed in fresh reduced-fat cheese by Juan et al. (2013), there was an inverse proportion between lentil milk and taste; as the amount of lentil milk increased, the taste became more unpleasant (the taste score was decreased). Similar results related to negative effect of lentil flours on the sensory properties of stirred yogurt was announced by Küçükçetin et al. (2012). The samples that contained both inulin and lentil milk at high level (L3I3) gave the highest taste score after the control sample.

The mean results indicated that the total acceptance of all samples was decreased toward the control sample. The highest acceptance was related to the second level of inulin (7%) in the absence of lentil milk (L1I2; Figure 3c). At the highest level of lentil milk (20%), the total acceptance was increased by raising the inulin value. Generally, a direct correlation was obtained between taste and total acceptance (P = 0.00, r = 0.81).

### 3.3 Texture profile analysis

The ANOVA results in Table 5 illustrate the effect of inulin and lentil milk on the textural parameters (firmness, cohesiveness, and gumminess).

Samples with 20% of lentil milk (L3I1, L3I2, and L3I3) had an incoherent and sandy texture that they were inapplicable for analysis by TPA (Figure 4). Although inulin improved texture of samples containing lentil milk, but at high level of lentil milk (20%), inulin was not able to improve the texture profile of samples.

According to Figure 5a, firmness was increased by increasing the inulin level, but there was no significant difference between the variant levels of inulin. Similar results were obtained by Hennelly et al. (2006). In their study, the hardness of cheese supplemented with inulin was quite higher than the control sample; also, the different
levels of inulin did not significantly affect the firmness of the cheese samples. There was no significant difference between samples which contained the second level of lentil milk solely or mixed with inulin (L2I1, L2I2, and L2I3).

The comparison of the mean results of the interaction effect between lentil milk and inulin on the cohesiveness showed that the increase in the inulin concentration had no considerable effect on cohesiveness; also, no significant difference was observed between samples supplemented by different levels of inulin and the control sample (Figure 5b). The cohesiveness of samples was decreased by increasing the lentil milk concentration.

As Figure 5c shows, a significant raise was observed in gumminess as the inulin concentration increased. The higher level of

**TABLE 3** Mean protein content of feta cheese (mean ± SD)

| Sample | Protein content (%) |
|--------|---------------------|
| L1I1 (control) | 18.23 |
| L1I2 | 18.93 ± 0.07 |
| L1I3 | 19.21 ± 0.08 |
| L2I1 | 20.27 ± 0.20 |
| L2I2 | 20.73 ± 0.09 |
| L2I3 | 21.04 ± 0.11 |
| L3I1 | 25.44 ± 0.03 |
| L3I2 | 25.91 ± 0.1 |
| L3I3 | 26.33 ± 0.02 |
### TABLE 4  ANOVA results: The effect of inulin and lentil milk on the sensory analysis

| Variable factors         | Degree of freedom | Mean squares (Msq) | Taste | Stiffness | Spread ability | T. acceptance |
|--------------------------|-------------------|--------------------|-------|-----------|----------------|---------------|
| Inulin                   | 2                 | 1.083ns            | 0.715ns | 0.674ns   | 0.095ns        |               |
| Lentil milk              | 2                 | 21.521***          | 96.507*** | 5.777**   | 18.271***      |               |
| Inulin × lentil milk     | 4                 | 5.042**            | 0.747ns | 4.944***  | 2.348***       |               |
| Error                    | 135               | 1.129              | 0.562 | 0.806     | 0.301          |               |

Abbreviation: ns, non-significant.  
**Significant (0.01 > P > 0.001).  
***Significant (P > 0.001).

**FIGURE 3**  (a) The effect of inulin and lentil milk on stiffness; (b) the effect of inulin and lentil milk on spreadability; (c) the effect of inulin and lentil milk on total acceptance (L1: 0% lentil milk; L2: 10% lentil milk; L3: 20% lentil milk; I1: 0% inulin; I2: 3.5% inulin; I3: 7% inulin)
gumminess was related to the samples with third level of inulin (7%) without lentil milk (L1I3). However, the addition of inulin increased the gumminess of the samples, but inulin in combination with 10% of lentil milk decreased the gumminess. Samples contained different levels of inulin besides 10% lentil milk had no significant difference compared to the control sample.

4 | DISCUSSION

A part of cow’s milk was replaced with lentil milk and inulin to produce cheeses with nine different formulations. The pH value in the samples containing the second level of lentil milk in the presence of inulin and without inulin was similar to the control sample, and there was no significant difference between samples. The pH value decreased with increasing the concentration of lentil milk up to 20%, which the lowest pH was observed in the sample containing the highest level of lentil milk (20%). This may be due to the presence of lentil milk which acidifies the samples. Also, Zare (2011) demonstrated that the lentil flour increased the acidity of yogurt samples. The amount of solid matter of the samples containing inulin without lentil milk was slightly increased compared to the control sample, but no significant difference was observed between these samples and the control sample. One of the most important factors that affect the solid matter of cheese is the absorption of water by proteins. The high amount of protein in lentil increases the water absorption capacity of the samples and prevents any water losses. Therefore, by increasing the level of lentil milk in presence or without inulin, the solid matter was decreased; also, it shows an increase in the moisture content of samples. As mentioned before, samples with 20% of lentil milk (L3I1, L3I2, and L3I3) had an incoherent and sandy texture due to the reduction of solid matter and subsequently increase the moisture content. Also, increasing the water-binding capacity of lentils by heating increases the moisture content of samples containing lentil milk. Therefore, inconsistent texture of samples containing high level of lentil milk may be related to this raise in the water-binding capacity of lentil seeds by heating.

In the case of protein, the protein content was increased by gradually enhancing the lentil milk and inulin content. Several studies were used the vegetable proteins such as soybean and peanut proteins as a partial or total replacement for casein in cheese production (Anonymous, 1989; Chen et al., 1979). Our results were in accordance with those of Haq et al. (2019), who studied the yogurt supplemented by lentil, which the protein content of samples were increased by raising the lentil flour. As mentioned before, lentil has high protein content than milk; also, the protein matrix becomes more compact due to the reduction of fat content by replacing a part of the cow’s milk with lentil milk (Karimi et al., 2015). The effect of inulin on cheese protein content was mentioned by Juan et al. (2013). They indicated the higher amount of protein in cheese containing inulin against both full-fat and reduced-fat cheeses. In their study, the addition of 5% inulin in milk resulted in maintenance of 3% of inulin in reduced fat cheese with inulin. The three mainly components of whey protein are β-lactoglobulin (85%), α-lactalbumin (10%), and bovine serum albumin (5%; Li et al., 2019), which inulin interacts with β-lactoglobulin due to the structure of β-lactoglobulin and capability to bind with hydrophobic compounds. Therefore, the high amount of protein in lentil milk and inulin becomes part of the milk protein and leads to an increase in the protein content in the cheese samples. The taste score of the control sample was higher than the other samples. Actually, the amount of fat in raw milk affects the taste of samples by releasing some fat-soluble compounds during chewing (McCarthy et al., 2016). Due to the replacement of a part of cow’s milk with lentil milk and inulin, the fat content of the samples decreased, which can affect the taste of samples. The highest sensory score among the

| Variable factors | Degree of freedom | Mean squares (Msq) |
|-----------------|------------------|-------------------|
| Inulin          | 2                | 550,408***        |
| Lentil milk     | 2                | 3,642,108***      |
| Inulin × lentil milk | 4    | 520,510***       |
| Error           | 9                | 15,808            |

Abbreviation: ns, non-significant.
*Significant (0.05 > P > 0.01).
**Significant (0.01 > P > 0.001).
***Significant (P > 0.001).
samples supplemented with lentil milk and inulin was belonged to the sample containing the third level of lentil milk and inulin, 10% and 3.5%, respectively. Inasmuch as, increasing the level of lentil milk reduced the taste score of the samples, so it seems that the sensory improvement may be the result of the presence of inulin. When inulin dissolved in water or milk, micro-crystals are formed that cannot be felt in the mouth but lead to a smooth or creamy structure which affects the taste of the product (Karimi et al., 2015). The cheese samples which contained a high level of lentil milk had a sandy texture. The water storage capacity of legumes increases with heating; these may be attributed to the breakdown of starch and denaturation of proteins; then, water molecules bind to hydroxyl groups of amylose and amylopectin by hydrogen bonds. Therefore, high moisture in cheese may result in decreasing the stiffness and create a sandy texture. Increasing the inulin concentration at a high level of lentil milk (20%) made the cheese texture more unsuitable. These may be related

**FIGURE 5** (a) The effect of inulin and lentil milk on firmness; (b) the effect of inulin and lentil milk on cohesiveness; (c) the effect of inulin and lentil milk on gumminess (L1: 0% lentil milk; L2: 10% lentil milk; L3: 20% lentil milk; I1: 0% inulin; I2: 3.5% inulin; I3: 7% inulin)
to the negative interaction between inulin and starch, because both of them have high water-binding capacity (Bishay, 1998) or/and high water storage capacity of lentil milk due to denaturation of lentil proteins during heating (Ettoumi & Chibane, 2015). Our observations were consistent with Guinee et al. (2004), who reported a decrease in firmness at high levels of plant proteins. As the part of cow’s milk was superseded with lentil milk, we expected that this cause decreasing in spreadability due to the decrease in fat levels. Lentil has a high emulsifying activity (37.17%) (Karaka et al., 2011). High emulsifier activity index of lentil and interaction between protein-polysaccharides (fiber) forms homogenous biopolymer films in the interface (Boye et al., 2009); hereupon, the spreadability of samples did not change. On the other side, when inulin is dissolved in water, a 3D gel network is formed by inulin’s micro-crystals, which directly affect the smooth and creamy structure of the product (Karimi et al., 2015). Juan et al. (2013) revealed that the hygroscopic nature of inulin leads to an increase in the water-binding and gel-formation activity in cheeses containing inulin. From the point of view of panellists, supplemented samples solely with lentil milk were very unacceptable, especially samples with high lentil content (20%). At the second level of lentil (10%), the addition of inulin increased the acceptance of the sample by panellists. The increase in the firmness of the samples by increasing the level of inulin may be due to the interaction between inulin and milk proteins; finally, inulin becomes a part of the protein structural network; these happen when inulin is present during fermentation and coagulation (Kip et al., 2006). Gibowski and Gibowska (2009) declared that the increase in inulin concentration up to 15% increased the shear stress value for whey protein solutions. Therefore, the increase in shear stress may lead to an increase in the hardness of samples. The decrease in the cohesiveness of the samples with an increase in lentil milk may be the result of an increase in the water-binding capacity of lentil milk during heating (Ettoumi & Chibane, 2015). As mentioned in the results, the gumminess decreased by increasing the lentil milk and increased in the presence of inulin. Inulin gels consist of 3D network of insoluble microstructure crystals that entrap a high amount of water in this network and make the product gummier (Bot et al., 2004). The fat content in lentils is slight (Kowieska & Petkov, 2003; Wang & Toews, 2011), and when part of cow’s milk is replaced with lentil milk, a reduction in fat content may affect the gumminess. Also, the negative interaction between inulin and lentil starch may be the cause of reducing gumminess in the presence of lentil milk and inulin together. The results of the present study showed that the plant sources especially lentil can be a good source to produce analogue cheese. Although lentil milk has a high water storage capacity and this increases by heating, using a lower amount of this crop as a replacer leads to better results, because a high concentration like 20% in this study results in undesirable texture. Also, inulin as a fat replacer and texture improver can be effective in different concentrations. The sample containing 10% of lentil milk and 3.5% inulin gave the better chemical and TPA results and also gained the most acceptances from the view point of panelists. Generally, dairy products with plant sources like vegetable cheeses can be a good alternative to the common cheeses; indeed, they have many health effects. More studies are needed to find new plant sources that can be used in dairy products.

CONFLICT OF INTEREST
The authors confirm that they do not have any conflict of interest.

AUTHOR CONTRIBUTION
Ziarati and Sawicka supervised the project. Moradi carried out the experiments. Ziarati and Moradi wrote the manuscript with support from Sawicka. All authors discussed the results and contributed to the final manuscript.

ETHICAL REVIEW
This product was produced in a completely sterile condition.

DATA AVAILABILITY STATEMENT
Data are openly available in a public repository tissues datasets with DOIs.

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**How to cite this article:** Moradi D, Ziarati P, Sawicka B. Physicochemical and rheological properties of lentil milk/inulin blend—A feta cheese analogue. *Legume Science*. 2021:e80. https://doi.org/10.1002/leg3.80