

Original article

Rheological, thermal and sensory properties of whey protein concentrate/pectin-fortified mashed potatoes made from dehydrated flakes

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Summary The effect of different amounts of whey protein concentrate (50–150 g kg⁻¹), low and high methoxyl pectin (5–15 g kg⁻¹) on the rheological, thermal, structural properties and sensory quality of mashed potatoes prepared from dried mashed potatoes flakes was investigated. The response surface technique was used to analyse the effects of whey protein concentrate and pectin simultaneously on the consistency index, flow behaviour index, apparent viscosity and Casson plastic viscosity. Both whey protein concentrate and pectin decreased the consistency of the mashed potatoes weakening its structure in all concentrations assayed. Results suggest that whey protein concentrate interacts with high methoxyl pectin through non-covalent interactions. Based on the sensory evaluation results, up to 100 g kg⁻¹ whey protein concentrate with 15 g kg⁻¹ of low methoxyl pectin and 15 g kg⁻¹ of high methoxyl pectin could be incorporated to dried mashed potatoes flakes without losing significantly the sensory quality of the product.

Keywords Food/feed fortification, functional properties, pectin, response surface methodology, rheology, sensory evaluation.

Introduction

Mashed potatoes (MP) are important segments of potatoes-based convenience food for both individual households and catering institutions (Lamberti *et al.*, 2004). It can be prepared directly from fresh potatoes or from the commercial product presented as dehydrated mashed potatoes flakes (DMPF). In both cases, it is a typical carbohydrate-rich food.

Whey protein concentrate (WPC) provides nutritional value and functional properties such as its ability to increase viscosity. Thus, changes in the viscoelastic properties of foods can be achieved using WPC. On the other hand, pectin are one of the most common sources of dietary fibre in human nutrition. Pectin can be divided into low methoxyl pectin (LMP) and high methoxyl pectin (HMP) according to the number of methoxyl groups in the molecule. The degree of esterification modifies many of the functional properties of pectin, such as the rheological behaviour and gelation (Serguschenko *et al.*, 2007); therefore, the addition of both types of pectin would allow obtaining products with different rheological properties.

During food processing, changes in the rheological properties may occur. Thus, rheological tests are a key

technique to understand the flow behaviour of MP. Taste and texture are important quality parameters for the consumer acceptance of MP. While a fluffy and medium-consistent texture is desired, pasty, gummy and sticky are negative attributes in any case (Lamberti *et al.*, 2004). The addition of WPC and pectin to DMPF could modify the sensory properties of the reconstituted product; thus, a sensory evaluation test should be carried out.

The appearance of final product is essential and a white background is preferred to yellow (Reis & Canevarolo, 2012). The yellowness index (YI) is a quality parameter that describes the colour change from clear or white towards yellow. Zhang *et al.* (2012) used the YI to characterise the colouration of UV-cured coatings. However, in certain foods, a slight yellow colour is often expected. MP is an example of food in which the yellow colour is usually accepted by consumers. The addition of WPC could enhance the yellowness of MP beyond the limits of the consumer's acceptance. Thus, the control of this parameter in MP supplemented with WPC and pectin is as important as the control of other physical properties.

There are few studies available in the literature concerning MP enriched with different components. Alvarez *et al.* (2010, 2011) studied the influence of freezing and cryoprotectant on the properties of MP

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and functionality of inulin/olive oil-based MP. Fernandez *et al.* (2008) analysed the effect of the addition of different biopolymers on the rheological properties of fresh and frozen/thawed MP. Kluge *et al.* (1979) patented DMPF with WPC, but the work is focused only on the sensory properties of the reconstituted product. Hence, no information is available concerning a commercial DMPF added with WPC and other component like pectin, and how the interactions between biopolymers affect the rheology, thermal, structural properties and sensory quality of the final product.

The objectives of this study were the use of response surface methodology (RSM) to study the simultaneous effect of WPC and pectin on the consistency index, flow behaviour index, apparent viscosity and Casson plastic viscosity of MP. The thermal, colour and sensory properties and protein solubility of MP prepared from DMPF with WPC and pectin, and the comparison with the standard commercial product were also studied to achieve a nutritional enhanced product keeping the texture and sensory characteristics

Materials and methods

Materials

WPC was a gift of Arla Foods Ingredients S.A. (Martinez, Buenos Aires, Argentina) and contained 777.1 g kg⁻¹ protein (N × 6.38), 57.4 g kg⁻¹ moisture, 27.7 g kg⁻¹ ash, 38.3 g kg⁻¹ lipids and 99.5 g kg⁻¹ lactose (estimated by difference). LMP (GENU pectin type 104, degree of amidation: 20%) with a degree of esterification of 27%, and HMP (GENU pectin type 105, rapid set) with a degree of esterification of 70%, was purchased from Gelfix S.A. (Buenos Aires, Argentina). DMPF was prepared with potatoes of the type Pampeana INTA by Conosud S.A. (Santa Fe, Argentina). Commercial butter was purchased from Mastellone Hnos. S.A. (La Serenisima, Gral. Rodriguez, Buenos Aires, Argentina). All chemicals used were of analytical grade.

Experimental design

A Box-Behnken design was obtained using the Statgraphics plus 5.1 software (SYSTAT, Inc., Evanston, IL, USA). RSM was used to analyse the simultaneous effect of WPC and pectin on the rheological parameters: flow behaviour index (*n*), consistency index (*K*), Casson plastic viscosity (η_C) and apparent viscosity (η_{app}) (responses). The three independent variables were as follows: WPC concentration (x_1), LMP concentration (x_2) and HMP concentration (x_3) were incorporated into the design and were analysed in 17 combinations. The coded and uncoded levels of the independent variables are shown in Table S1. The

central point of the design was repeated five times to calculate the reproducibility of the method.

The effect of these three independent variables x_1 , x_2 and x_3 on the responses (*Y*) was modelled using the second-order polynomial response surface. The equation derived using RSM for the prediction of the response variables is as follows:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (1)$$

where β_0 is the value of the fixed response (*Y*) at the point 0 g kg⁻¹ of WPC and 0 g kg⁻¹ of pectin; β_1 , β_2 and β_3 are the linear; β_{11} , β_{22} and β_{33} are the quadratic; and β_{12} , β_{13} and β_{23} are the interaction regression terms.

Sample preparation

Samples without WPC or pectin (control) were prepared according to the instructions of the manufacturer. On the other hand, the rest of the samples were prepared in the same way, but adding the WPC (0, 5, 10 and 15 g kg⁻¹), LMP and/or HMP (0, 5, 10 and 15 g kg⁻¹) as a dry powder together with DMPF. The three biopolymers were added according to the Box-Behnken design mentioned above. As suggested by Fernandez *et al.* (2008), range-finding experiments were performed at the outset of this study to ascertain the maximum acceptable amount of each biopolymer that could be added to the MP on the basis of flavour, viscosity and colour. To avoid gelatinisation or protein denaturation, samples for DSC were prepared at a room temperature between 25 and 30 °C and were left for 3 h before analysis to ensure a complete hydration.

Rheological measurements

The rheological properties were investigated using a RS 600 controlled stress rheometer (Haake, Karlsruhe, Germany) to conduct steady shear experiments using a 1.5-mm gap parallel plate geometry. Samples were placed on the lower plate thermostated at 55 °C, which is the preferred temperature for consumption of MP (Alvarez *et al.*, 2009). After loading the sample, a waiting period of 3 min was used to allow the sample to recover and to reach 55 °C. Solid vaseline was added around the plate edges to prevent dehydration. The equipment was driven through the Haake software Rheowin 3.30 Job Manager. Flow behaviour is often studied with the Ostwald de Waele and Casson models (Benezech & Maingonnat, 1994). Sample flow was measured by recording shear stress values when shearing with an increasing shear rate from 0 to 200 s⁻¹. Apparent viscosities values at a shear rate of

50 s^{-1} (η_{app}) were calculated at the rising curve. Experimental data were fitted to the Ostwald de Waele and Casson models using shear stress data (Alvarez *et al.*, 2010). The flow behaviour index (n) and the consistency index (K) were obtained from the de Waele model and the Casson plastic viscosity (η_C) from the Casson model. Two or more independent replicates of each combination were analysed.

Colour

Superficial colour was measured with a Chroma meter CR 300 Minolta (Osaka, Japan), and Hunter parameters were determined to calculate the yellowness index (YI):

$$YI = 142.86b^*/L^* \quad (2)$$

where L^* is the lightness of the colour and b^* its position between yellow and blue (Fernandez *et al.*, 2008). Values are the average (\pm standard deviation) of three independent replicates

Differential scanning calorimetry (DSC)

A differential scanning calorimeter (DSC Q100; Thermal Analysis Instruments, New Castle, DE, USA) calibrated with indium was used. Samples of 8–15 mg of MP with or without WPC or pectin were placed in aluminium DSC hermetic pans. An empty double pan was used as reference. Sample and reference were heated between 25 and 120 °C at a heating rate of 10 °C min^{-1} . Thermograms were analysed using the TA Instruments Universal Analysis 2000 software version 4.2E (ACD labs, New Castle, DE, USA).

Determination of the protein solubility

The protein solubility assays were performed according to techniques described in previous works by the subscribing authors (Yamul & Lupano, 2003, 2005; Cassiani *et al.*, 2013). Three independent extractions were carried out with each solvent and average values (\pm standard deviation) were reported. The intermediate levels of 100 g kg^{-1} of WPC and 10 g kg^{-1} of pectin were assayed.

Sensory analysis

Three independent sessions with a difference of 7 days among each other were carried out to study the effect of the addition of WPC and WPC and pectin together. Panellists were recruited among workers of the university who declared that they were all regular consumers of this kind of products and they were not hungry at the moment of the test. The test samples at 55 °C were presented to 72 non-trained panellists in plastic

containers coded with a three-digit number according to a randomised complete block design. The order of presentation was balanced and randomised to eliminate contrast effect and positional bias. All tests were performed between 11 and 12 AM. The experimental environment was kept constant for all sessions and no outside influences were allowed to interfere with the subject's assessments of the product (smells, music, temperature, etc.). The panellists evaluated all the samples for colour, taste, texture and overall acceptability based on a nine-point hedonic scale (with 8 cm) labelled at each anchor: (left anchor: 1 = dislike extremely; right anchor: 9 = like extremely) as suggested by Alvarez *et al.* (2011). Results were expressed as a percentage of the total scale distance. Mineral water was used to clear their palates between samples. Samples evaluated are described in Table S3.

Microstructure

Microstructure was observed with a LEICA TCS SP5 Confocal Laser Scanning Microscopy (Leica Micro systems, Baden-Württemberg, Germany) configured with an inverted microscope (Leica Microsystems). A He/Ne visible light laser at a power of 30% was used. Objective lens: 63 \times 1.4 of numerical aperture with a zoom of 1.7. A fluorescent probe of 0.32 ml of 0.01% Rhodamine B/0.8 g protein (Lutz *et al.*, 2009) was used for non-covalent labelling of proteins. The probe was added at the moment of the MP preparation, to facilitate the dye diffusion. The excitation wavelength was 543 nm and the emission wavelengths between 557 and 626 nm were collected. Digital image files were acquired in 1024 \times 1024 pixel resolution and analysed with LAS AF LITE software (Leica Microsystems).

Statistical analysis

An analysis of variance (ANOVA) was conducted separately on the dependent variables studied (protein solubility, YI, colour, taste, texture and overall acceptability) in a one-way factorial design. The least significant differences (LSD) were calculated to compare the means at a level of 95% using the Fisher test. All the statistical procedures were computed using the SYSTAT 12 statistical software (SYSTAT, Inc., Evanston, IL, USA).

Results and discussion

Model fitting from RSM

The independent and response variables were fitted to the second-order model equation (Eq. 1). The coefficient of determination or R^2 is the proportion of variation in the response attributed to the model rather

than to random error and was suggested that for good fit model, R^2 should be at least 80% (Gan *et al.*, 2007). The regression coefficients (β_i) and the coefficients of determination (R^2) of the equation derived using RSM for the prediction of the response variables are shown in Table S1. Results showed that the models for all the response variables were highly adequate because they had satisfactory levels of R^2 of more than 80%. These results indicate that a high proportion of variability can be explained by the data; therefore, the response surface models developed were adequate. To aid visualisation, the response surfaces are presented in three-dimensional graphs (Fig. S1), showing the effect of two independent variables on a response variable, keeping constant in the intermediate level (0) the third independent variable.

Figure S1 (a–l) depicts that the K , η_{app} , n and η_C of the MP depend on the amount of WPC, LMP and HMP as its linear and quadratic effects were negative in almost all cases analysed at $P < 0.05$ (Table S2). These results suggest that the increase in hydrocolloids content softens the MP. Similar results were obtained by Alvarez *et al.* (2010) with the addition of inulin to MP. Moreover, Fernandez *et al.* (2008) suggest that the flow behaviour of potato starch and biopolymers like whey proteins and pectin rules the pseudoplastic behaviour of MP. Results also suggest that the main effect on the rheological parameters is caused by the addition of WPC as reflected by the higher values of the regression coefficients (β_1 and β_{11}). On the other hand, LMP exhibited the lowest regression coefficients (β_2 and β_{22}) suggesting that the effect of this component is not as important as the others.

Interactions between whey proteins and pectin in MP are important as they would influence the physical structure of the product modifying the texture and the rheological properties. Proteins and polysaccharides, such as pectin, having opposite electrical charges can form electrostatic complexes (Jones *et al.*, 2010). The pH of MP is 5.5–6.0 and is above the isoelectric point of whey proteins and the pKa of carboxyl groups of pectin. Thus, both biopolymers are negatively charged and the electrostatic repulsion should prevent any complex formation reducing the physical interaction between them. As was expected, interactions between WPC and LMP (β_{12} , Table S2) were not significant ($P > 0.05$); however, significant interactions ($P < 0.05$) were observed between WPC and HMP (β_{13} , Table S2). These results could be explained taking into account that the ionic charge of HMP is lower than the ionic charge of LMP due to the higher number of methoxyl groups in HMP. Hence, some interactions could be possible between HMP and the hydrophobic and uncharged zones of the whey proteins. On the other hand, no significant interactions ($P > 0.05$) were observed between LMP and HMP (β_{23}).

The value of the flow behaviour index (n) is a measure of deviation from Newtonian flow and indicates pseudoplastic behaviour. For shear thinning fluids, $n < 1$, whereas for Newtonian fluids, $n = 1$ (Rohm, 1993). Figure S1 (j–l) shows that WPC, HMP and LMP increases the n with linear positive effects and negative quadratic effects, suggesting that these components reduce the pseudoplasticity increasing the Newtonian fluid-like behaviour.

Thermal characteristics

Figure S2 shows the thermograms obtained when MP with different WPC and pectin content were heated in a DSC apparatus. No peaks for starch gelatinization were observed in all samples analysed. These results are in agreement with those obtained by Lamberti *et al.* (2004) who studied the starch transformation in the production of potatoes flakes. Preheating process of fresh potatoes in tap water at 70 °C is usually carried out during DMPF production. According to Lamberti *et al.* (2004), this process would be sufficient to gelatinise the starch. Hence, starch would be gelatinised before the DSC run and no endothermic transition was observed (endotherm *a*). No peak is observed in endotherm *b* because endothermic transitions for HMP are only observed at temperatures above 200 °C (Einhorn-Stoll *et al.*, 2007; Einhorn-Stoll & Kunzek, 2009). The peak (II) showed in the endotherm *c* would correspond to whey protein denaturation, which occurs around 87 °C. The endotherm *d* showed an endothermic transition (I) corresponding to LMP, in agreement with results obtained by Gilsenan *et al.* (2000). No interactions between LMP and whey proteins were observed as both peaks of endotherm *e* are separated and at the same temperature of the biopolymers alone (endotherms *c* and *d*). These findings confirm the results of the RSM analysis in which no significant interactions were observed between WPC and LMP (β_{12}). Jones *et al.* (2010) found that LMP increased the thermal denaturation temperature of β -lactoglobulin, the major whey protein, but these authors carried out the experiments at pH 4.75. At this pH, whey proteins are positively charged and, thus, interactions with LMP are expected.

Protein solubility

The analysis of protein solubility using different media that disrupt different kind of bonds allows studying the interactions among whey proteins, potato starch and pectin. The protein solubility of the protein constituents of MP is shown in Fig. S3. As it was mentioned in section 3.1 at the pH of MP, all the biopolymers are negatively charged and the electrostatic repulsion should prevent any complex formation. The protein solubility in samples

2 and 3 did not change significantly ($P > 0.05$) with all the extraction solutions assayed suggesting that there are no strong interactions among the biopolymers. On the other hand, the protein solubility in sample 4 is lower than in samples 2 and 3 when DW and B were used suggesting that whey proteins could interact with HMP. However, it is not an electrostatic interaction as the protein solubility in DW and B is not significantly different ($P > 0.05$). The protein solubility in sample 4 with BS is higher than in others indicating that non-covalent interactions, such as hydrophobic and hydrogen bond, are involved between WP and HMP. These results are in agreement with the results mentioned in RSM analysis section and thermal characteristics section.

Sensory analysis

The first session of sensory analysis was carried out to determinate the maximum level of WPC, which could be incorporated without losing significantly sensory quality. Statistical results shown in Table S3 indicate that the mean values for each sensory attribute of samples without (control, standard commercial sample) or with 50 g kg^{-1} WPC were not significant ($P > 0.05$). On the other hand, mean value differences in the sensory attributes at 100 and 150 g kg^{-1} WPC were significant ($P < 0.05$) suggesting a decrease in the sensory quality of the product at these WPC levels. Except for the colour at 100 g kg^{-1} WPC in which no significant differences ($P > 0.05$) were observed. The decrease in the sensory texture coincides with a decrease in the η_{app} , K and η_C caused by the addition of WPC, suggesting that a softer MP is not acceptable by consumers. These correlations between sensory and instrumental measurements could be a useful tool for the food industry as sensory analysis is time and cost demanding. However, in the present case, a deeper statistical analysis should be performed to correlate the rheological parameters with the sensory texture. Similar results were obtained by Asadinejad *et al.* (2005) with the substitution of milk solids by WPC in an ice cream and Kluge *et al.* (1979) with a similar system. The off-flavour in samples with more than 50 g kg^{-1} WPC could be due to the lactose and lipids from WPC that are susceptible to chemical reactions (Morr & Ha, 1991).

Based on these results, the second session was carried out. Results in Table S3 indicate that the mean values for each sensory attribute of samples without WPC or pectin (control, standard commercial sample) or with 50 g kg^{-1} WPC combined with 10, 20 or 30 g kg^{-1} of pectin were not significant ($P > 0.05$), suggesting that pectin did not affect the sensory quality of the product at the concentrations assayed. The effect of pectin on the rheological parameters is not as important as the

effect of WPC according to the values of the regression coefficients (β_2 , β_3 , β_{22} and β_{33}) suggesting that panelists could not perceived the little differences observed instrumentally. Moreover, the addition of pectin did not modify significantly the YI of the product (Table S4).

Results of the third session in Table S3 suggest that pectin counteracts the decrease in the sensory quality at 100 g kg^{-1} WPC. Pectin probably masks the off-flavour of these products, whether by interacting with them or by competing with them for the gustatory receptors, or by other unknown mechanism. Nevertheless, more research is needed to establish the molecular basis of this effect.

Colour measurements

The amount of WPC was a significant factor ($P < 0.05$) for the YI of the MP as suggested by the results of Table S4. On the other hand, the addition of pectin (LMP or HMP) did not affect significantly ($P > 0.05$) the YI of the product. Despite the increase in the YI as WPC content increased, results of the sensory evaluation (Table S3) indicated that the colour of samples in the range of $50\text{--}100 \text{ g kg}^{-1}$ of WPC showed no significant differences ($P > 0.05$) with the colour of samples without this biopolymer. These results suggest that panellist did not perceive the colour differences between 50 and 100 g kg^{-1} WPC observed instrumentally.

Microstructure

Figure S4 depicts the microstructure of MP observed with confocal laser scanning microscopy. The Fig. S4a of the control, without either WPC or pectin, shows that the MP manufacturing process damages all the potato cell structure yielding a smooth structure. On the other hand, whey proteins are visualised (Fig. S4b) in a separate phase inserted in the microstructure of the MP making it more rough. This result is reflected in the decrease in the sensory texture when WPC is added (Table S3). The changes observed in the microstructure produced a softer system with a lower viscosity. As can be seen in Fig. S4c, the addition of pectin counteracts the effect of WPC smoothing the microstructure and confirming results of the sensory evaluation (Table S3).

Conclusions

WPC and pectin changes the flow behaviour of MP towards a more liquid-like product. The RSM model developed could be used to estimate, within the specified range of work, the effect of different factors on the rheological properties of MP. The addition of WPC and pectin to DMPF would become the product

particularly attractive to people pursuing a better nutritional balanced food.

The formulations with 50 or 100 g kg⁻¹ WPC combined with 30 g kg⁻¹ pectin allow to obtain a product with the same functionality of the product alone and with the advantages of not having to use milk, becoming the formulation a really 'ready to cook food'.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Response surfaces for the combined effects of WPC, LMP and HMP on the rheological properties of the MP.

Figure S2. DSC thermograms of MP with WPC and pectin.

Figure S3. Solubility of the protein constituents of mashed potatoes (MP).

Figure S4. Microstructure of mashed potatoes with whey proteins and pectin observed by confocal laser scanning microscopy.

Table S1. Coded and coded levels of the independent variables used in the experiment.

Table S2. Coefficients of determination (R^2) and estimated regression coefficients (β_i), of the fitted second-order polynomial for the response variables: Consistency index (K); apparent viscosity (η_{app}); Casson plastic viscosity (η_c) and flow behaviour index (n).

Table S3. Mean values and standard deviation for each sensory attribute at different WPC and pectin content.

Table S4. Yelowness index (YI) as a function of mashed potatoes composition.