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Gluten-free bread formulated with *Prosopis ruscifolia* (vinal) seed and corn flours

C. BERNARDI, H. SÁNCHEZ, M. FREYRE & C. OSELLA

Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina

Abstract

Vinal (*Prosopis ruscifolia*) is a wild leguminous tree found widely in the north of Argentina. Like other *Prosopis*, vinal can grow under extreme temperatures, in poor soils and can tolerate high saline conditions. Taking into account the high protein and gums contents of vinal seeds, a gluten-free bread was developed including them and corn flour. A central composite design involving vinal seed flour/corn starch ratio (X1) and corn flour/corn starch ratio (X2) was used, and second-order models for specific volume (Y1) and bread score (Y2), evaluated by an expert panel, were employed to generate response surfaces. In the optimum zone of response surfaces, a product with higher protein content (5.2 g/100 g) than gluten-free breads found in local commercial markets was obtained. Also, an interesting antioxidant activity (115 mg ascorbic acid equivalent/100 g) was found in optimized gluten-free bread.

Keywords: *Prosopis sp., Prosopis ruscifolia, gluten-free bread, celiac disease*

Introduction

Vinal (*Prosopis ruscifolia*) is a leguminous tree found widely in the north of Argentina, covering 2 million ha, especially in Formosa province, and having almost no industrial use. Like other *Prosopis*, vinal can grow under extreme temperatures, in drought and poor soils as it can tolerate high saline conditions (Freyre et al. 2002). Its fruits, which are indehiscent pods, can be separated into exocarp, mesocarp (pulp), endocarp and seeds. Vinal seeds represent approximately 16% of the pods but they are under-utilized nowadays and, if it is taken into account that this species is found in a region characterized by a very low-income people, the importance of this contribution could have been evaluated (Freyre et al. 2002).

Celiac disease is a pathology increasingly detected not only in Argentina but all over the world. Celiac patients suffer a syndrome characterized by damage of the small intestine mucosa caused by ingestion of prolamine of wheat and the related cereals (oats, rye and barley), the only certain remedy being the total omission of gluten from the diet (Gallagher et al. 2004). There are many publications dealing with gluten-free product development (Sánchez et al. 2002; Schober et al. 2005). Wheat starch have been used to replace wheat flour but it was found that many individuals are so sensitive that they cannot tolerate even small amounts of gliadin and it became necessary to look

Correspondence: C. Bernardi, Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, 1° de mayo 3250, 3000 Santa Fe, Argentina. cbernard@fiq.unl.edu.ar

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for non-wheat cereal ingredients (Gallagher et al. 2004). Some starches, such as corn or cassava, can be consumed by celiac people but they have technological problems in bread-making because of their absence of gluten. Lacking the gluten network, gas holding is more difficult so gums are used to replace the elastic and extensible properties of gluten in this type of bread-making. It is important to mention that despite an acceptable gluten-free bread being obtained, there is a lack in proteins in those formulations and consequently a deficiency in the celiac patients' diet. Soy flours and soy protein concentrates were used by Ranhotra et al. (1975) to improve grain and texture but soy could not be used at high levels without severely decreasing bread quality, so it could be interesting to find a protein source to be used in this kind of bread.

The aim of the present study was to use *Prosopis ruscifolia* seed flour to formulate gluten-free bread knowing its high protein (38–40 g/100 g) and dietary fiber (50–53 g/100 g) contents, gums being a great proportion of the latter (Freyre et al. 2002). Optimal amounts of corn starch, vinal seed and yellow corn flours were established statistically to obtain an acceptable gluten-free bread. A final optimized bread was obtained and analyzed in order to evaluate the proximate composition. As vinal seed flour proved previously to be rich in polyphenols, the total polyphenols and antioxidant activity were also analyzed taking into account that there is much scientific evidence indicating the paramount role of food antioxidants in the prevention of different types of cancer and coronary diseases and that there is a growing demand of functional foods (Marnett 2000). As there are no literature data about the biological value of vinal proteins, essential amino acids were analyzed and the corresponding chemical scores for each amino acid were calculated.

Materials and methods

Preparation of P. ruscifolia seed flour

P. ruscifolia (vinal) pods were collected in Formosa province (Argentina), packed into bags and transported to the laboratory. The pods were dried at 50°C for 72 h in a convection electric oven (Bioelec, Santa Fe, Argentina) to 4 g/100 g moisture before milling. Milling was carried out in a disc mill (Pulverizer type; BICO INC, Burbank, CA, USA), obtaining a mixture of pod fractions. The fraction containing the seeds covered by endocarp was taken and milled in a laboratory refiner (Feeder Industries Inc., Muncy, PA, USA) in order to separate the seeds. After that the seeds were separated pneumatically and then ground in a laboratory mill (Cyclotec; UDY Corp, Fort Collins, CO, USA) in order to obtain a particle size <0.25 mm.

Proximate analysis

The moisture, fat and crude protein contents were determined according to AOAC (1995) methods in optimized bread, corn and vinal seed flours.

Gluten analysis

Gluten was determined not only in vinal seeds and corn flours but also in optimized bread. A commercial RIDASCREEN Gliadin ELISA kit (R.Biopharm AG, Darmstadt, Germany) was utilized, of which the detection limit is 5 ppm gliadin.

Total gum analysis

Gums were analyzed in vinal seed flour by alcohol precipitation. The content was determined by analyzing total sugars in the precipitate by the method of Dubois et al. (1956), expressing results as grams of mannose per 100 g.

Polyphenols analysis

Polyphenols were analyzed by the Folin–Denis method (Chavan et al. 2003). A 200 mg sample of vinal seed flour, corn flour or optimized gluten-free bread was added to 10 ml methanol. The samples were agitated at 225 rpm on a shaker at ambient temperature for 20 min and filtrated. An aliquot of 1 ml was taken from the filtrate, to which 0.5 ml Folin–Denis reagent and 1 ml saturated Na_2CO_3 were added and incubated for 30 min after addition. The absorbance was determined at 760 nm. A calibration curve was established using catequine as standard (Sigma Aldrich, St. Louis, MO, USA).

DPPH assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method has been extensively applied for the study of the antioxidant activity of foods, and presents the advantage of using a stable and commercially available free radical ($DPPH^{\cdot}$) (Leong and Shui 2002; Lim et al. 2007). The free radical scavenging activity of vinal seed flour, corn flour or optimized gluten-free bread were evaluated by measuring the decrease in absorbance of methanolic DPPH solution at 517 nm in the presence of methanolic extracts (Brandt-Williams et al. 1995). The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for 30 min. The amount of sample extracted into 1 ml solution necessary to decrease the initial DPPH concentration by 50% (IC_{50} value) was derived from the percentage disappearance versus concentration plot (concentration here means milligrams of sample extracted into 1 ml solution). The results are also expressed as the ascorbic acid (AA) equivalent antioxidant capacity (AEAC) (Lim et al. 2007):

$$AEAC = (1C_{50(AA)} / IC_{50(sample)} \times 10^5$$

The $IC_{50(AA)}$ value was determined to be $3.98 \pm 0.38 \times 10^{-3}$ mg/ml based on 10 determinations.

Amino acid analysis

Essential amino acids were determined according to Adams (1974). Samples were subjected to acid hydrolysis in the presence of 6 M HCl at 105°C for 24 h. Sulfur-containing amino acids were determined separately in 6 M HCl after oxidative hydrolysis (formic acid + hydrogen peroxide, 9:1 v/v, 20 h at 4°C). Tryptophan was determined according to the method described in AOAC (1995). Amino acid determinations were expressed on a milligram per gram of protein basis.

Estimation of nutritive values of vinal seed amino acids

Each essential amino acid's chemical score was calculated on the basis of a procedure described by Biel et al. (2009) based on a comparison of the concentration ratio of the amino acid content to that of this amino acid in a standard a_s (chemical score = $a_i/a_s \times 100$). The standard used was a food protein composition (FAO/WHO 1990).

Bread-making

The solid ingredients of corn flour (8.08 ± 0.45 g protein/100 g, 5.2 ± 0.4 g fat/100 g, 4.9 ± 0.2 g moisture/100 g, particle size <0.25 mm, gluten-negative) and vinal seed flour (39 ± 2.3 g protein/100 g, 5.3 ± 0.4 g moisture/100 g and 5.8 ± 0.2 g fat/100 g), at established experiment design levels, plus corn starch (PERLA cornstarch, 1.6% protein [%N × 6.25], 70°C gluten-negative gelatinization temperature; Industrias de Maíz S.A., Buenos Aires, Argentina) up to 100 g were added to 20 g fat (oleomargarine, melting point 36°C; Molinos Río de la Plata S.A., Buenos Aires, Argentina), 18 g sugar, 15 g yeast and 4 g salt. Then those ingredients were mixed with water at 400 rpm for 1 min and at 600 rpm for 2 min, in each experiment. Water was adjusted in order to obtain suited batters and was the same amount in all experiments (200 ml). Following Sánchez et al. (2002), doughs were then placed in greased bread pans and proofed at 27°C and 80% humidity until samples doubled their volumes (approximately 30 min). Breads were baked at 210°C for 40 min without steam. An apparatus was used to control proofing that consisted of a glass cylinder (75 mm in height, 45 mm i.d.) with a tight-fitting plastic piston that rises during proofing.

Volume measurement and sensory evaluation

The specific volume (cm^3/g bread) was determined 60 min after baking by millet-seed displacement, and then breads were evaluated by an expert panel of three individuals with more than 20 years of experience in baking, as in Sánchez et al. (2002). The samples were served as slices, at the same time. One slice of bread, identified by code number, was served to each panelist under normal (daylight) illumination. Each expert scored each sample once on three different occasions. As recommended by Sánchez et al. (2002) and Pyler (1973), the parameters evaluated and the maximum scores were: volume, 15; crust, 15; texture, 15; crumb color, 10; crumb grain, 10; aroma, 15; and taste, 20. The obtained final value was called the bread score.

Experimental design

Two responses were measured for runs: the specific volume (Y_1) and bread score (Y_2). The variables chosen, in order to be independent, were X_1 (corn flour/corn starch) and X_2 (vinal seed flour/corn starch). For each run, corn and vinal flour amounts were selected according to experimental design in order to attain a sum of corn starch plus corn and vinal flours reaching a grand total of 100 g before the addition of the remainder solid ingredients, as was mentioned in the Bread-making section. Each variable to be optimized was coded at five levels: –1.414, –1, 0, 1 and 1.414 (Table I). The selection of extreme levels was based on previous studies by the authors.

A central composite design, shown in Table II, was arranged to allow for fitting of a second-order model. The star points were added to the factorial design to provide for estimation of curvature of the model (Montgomery 1991). Six replicates at the

Table I. Variables and their levels for central composite design.

Uncoded variable	Coded variable					
	Variable	−1.414	−1	0	1	1.414
Seed vinal flour/corn starch	X_1	0.06	0.1	0.2	0.3	0.34
Corn flour/corn starch	X_2	0.8	1	1.5	2	2.2

center of the design were used to allow for estimation of the pure error at sum of the square.

Statistical analysis

Means and standard deviations were calculated for the proximate composition, polyphenols and antioxidant activity.

A software package (Statgraphics Plus 7.1; Manugistics Inc., Rockville, MD, USA) was used to fit the second-order models and generate response surface and contour plots. The model proposed for each response was:

$$Y = b_o + b_1 X_1 + b_2 X_2 + b_{11} X_1^2 + b_{22} X_2^2 + b_{12} X_1 X_2$$

where b_0 is the value of the fitted response at the center point of the design—that is, point (0,0)—b_1 and b_2 are the linear regression terms, b_{11} and b_{22} are the quadratic regression terms, and b_{12} is the cross-product regression term.

Tests to verify that the residues satisfy the assumptions of normality, independence and randomness were also evaluated. Significance was accepted at $P \leq 0.05$.

Table II. Central composite design arrangement and responses.

Run	Coded variable level[a]		Response[b]	
	X_1	X_2	Y_1	Y_2
1	1	−1	1.95	58
2	−1	−1	1.55	43.7
3	1.414	0	2.06	60
4	−1.414	0	1.52	35
5	0	−1.414	1.95	50.5
6	1	1	2.05	70
7	−1	1	1.52	35
8	0	1.414	2.05	60
9	0	0	2.15	70
10	0	0	2.04	65
11	0	0	2.14	68
12	0	0	2.11	70
13	0	0	2.09	70
14	0	0	2.05	65

[a]X_1 = vinal seed flour/corn starch; X_2 = corn flour/corn starch. [b]Y_1 = specific volume (cm^3/g); Y_2 = bread score.

Validation

For validation, some additional confirmatory experiments were performed. The bread score was determined and compared with predicted values. Differences between results and predicted values were determined by *t*-test analysis. Significance was accepted at $P \leq 0.05$.

Results and discussion

Vinal seed and corn flour analysis

Gums (galactomannans) represented 51 ± 2.2 g mannose/100 g vinal seed flour. Here it is important to mention that the gum content could contribute to replace the elastic and extensible properties of gluten in this type of bread-making.

As gluten-free commercial bread usually has a lack of proteins, vinal seed and corn flours could contribute them in our case. It is important to keep in mind that gluten-free breads are not generally enriched/fortified and are frequently made from starches so they may not contain the same levels of nutrients as the gluten counterparts they intended to replace. Therefore, uncertainty still exist as to whether celiac patients living on a gluten-free diet are ensured a nutritionally balanced diet (Gallagher et al. 2004).

The amino acid profile along with the essential amino acid scores in vinal seeds flour are presented in Table III. Vinal seed flour contains a relatively high amount of the analyzed essential amino acids. Similar levels of valine, isoleucine, threonine, hystidine, phenylalanine + tyrosine with remarkable lysine content were found in other *Prosopis* sp. seeds (Felker and Bandurski 1977; Marangoni and Alli 1988; Barba de la Rosa et al. 2006). As lysine is limited in most cereals, it may be possible to use vinal seed flour to improve the protein quality present in our gluten-free bread containing corn flour (Del Valle et al. 1983).

Table III. Amino acid composition and essential amino acid chemical scores in vinal seed flour (mg/g protein).

	P. ruscifolia seed	Essential amino acid score	FAO reference[a]
Alanine	57.3		
Arginine	51		
Aspartic acid	68.4		
Glutamic acid	284		
Glicina	59.2		
Histidine	13	68	19
Isoleucina	36	128	28
Leucina	99	150	66
Lysine	59	101	58
Methionine + cystine	12	48	25
Proline	13		
Phenylalanine + tyrosine	67	106	63
Tryptophan	8	72	11
Threonine	29	85	34
Serine	78		
Valine	46	131	35

[a]Data from FAO/WHO (1990).

Polyphenols proved to be in vinal seed and corn flours (ingredients that could contain them) at 1,955 ± 68 mg catequine equivalents/100 g and 43 ± 2 mg catequine equivalents/100 g, respectively. At the same time, antoxidant activity results were 1,300 ± 235 mg ascorbic acid equivalent/100 g for vinal seed flour but < 30 mg ascorbic acid equivalent/100 g for corn flour.

Gluten-free bread

Table II presents shows the central composite design arrangements and responses for the established points.

The assumptions underlying the analysis of variance test were investigated. A normal probability plot of residuals against estimated values for the response and a plot of residuals against random order of runs revealed that the residuals satisfied the assumptions of normality, independence and randomness. Therefore, the analysis of variance (Table IV) was performed and the residual (total error) was separated into lack of fit and pure error to determine whether the model appeared to fit the data or not.

Analyses of variance for specific volume (Table IV) show that only terms related to vinal seed flour/corn starch were significant. It is important to remember that vinal seed contains an interesting gum content that contributes to the bread specific volume retaining gas bubbles in the bread matrix. The coefficient of determination was satisfactory and equal to 0.933. On the basis of all these tests, the proposed model was accepted and specific volume was considered to be predicted by substituting coded variables:

$$\text{Specific volume}\,(Y_1) = 2.0966 + 0.2117X_1 + 0.02643X_2 - 0.1852X_1^2 - 0.08021X_2^2 + 0.0325X_1X_2$$

Analyses of variance for the bread score (Table IV) shows that all linear and quadratic terms were significant ($P < 0.05$), with the exception of the linear term for X_2 ($P = 0.06$).

Table IV. Analysis of variance for specific volume (Y_1) and bread score (Y_2).

		Sum of squares	
	Degrees of freedom	Y_1	Y_2
X_1 (vinal seed flour/corn starch)	1	0.3587*	895.816*
X_2 (corn flour/corn starch)	1	0.0056	35.0076
$X_1 \cdot X_2$	1	0.0042*	107.123*
X_1^2	1	0.2533*	764.533*
X_2^2	1	0.0048	293.094*
Lack of fit	3	0.0366	37.326
Pure error	5	0.01033	30.0
R^2		0.933	0.968

*Statistical significance: $P \leq 0.05$.

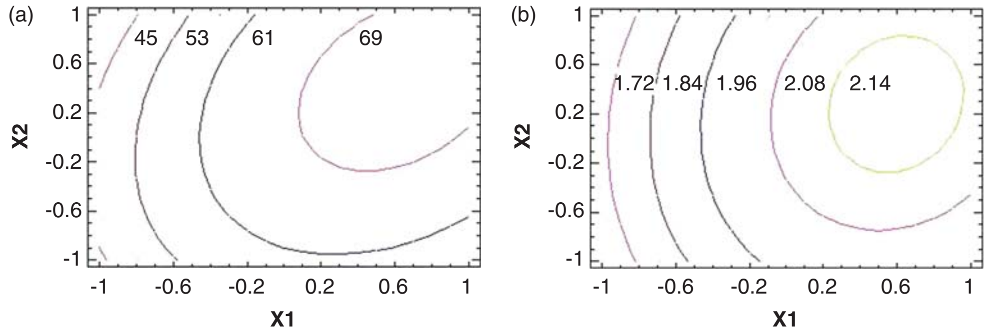

Figure 1. Contour plots of vinal seeds flour/corn starch against corn flour/corn starch ratios present in gluten-free formulations at constant values of (a) bread score and (b) specific volume (cm^3/g).

In this case, the second-order polynomial also did not show a significant lack of fit ($P < 0.05$) and the coefficient of determination was adequate and equal to 0.968, being the proposed model for the bread score:

$$\text{Bread score } (Y_2) = 68.0 + 10.5819X_1 + 2.092X_2 - 10.175X_1^2 - 6.3X_2^2 + 5.175X_1X_2$$

Contour plots of response surfaces are shown in Figures 1 and 2. As can be seen, not only the maximum bread score but also the specific volume were near the center points; coded values $X_1 = 0$ and $X_2 = 0$ correspond to uncoded variables: X_1 = seed vinal flour/corn starch = 0.2 and X_2 = corn flour/corn starch = 1.5; using the models calculated, the specific volume and bread score levels were 2.09 and 68, respectively.

A verification experiment at the conditions chosen, in what proved to be the optimum zone and consisting of four repeated runs, was performed. Chosen conditions were X_1 = 0.2 = vinal seed flour/corn starch, X_2 = 1.68 = corn flour/corn starch (expressed as uncoded variables), which corresponded to the formulation 58.33g corn flour, 6.94 g vinal seed flour and 34.72 g corn starch added to 20 g fat, 28 g sugar, 15 g

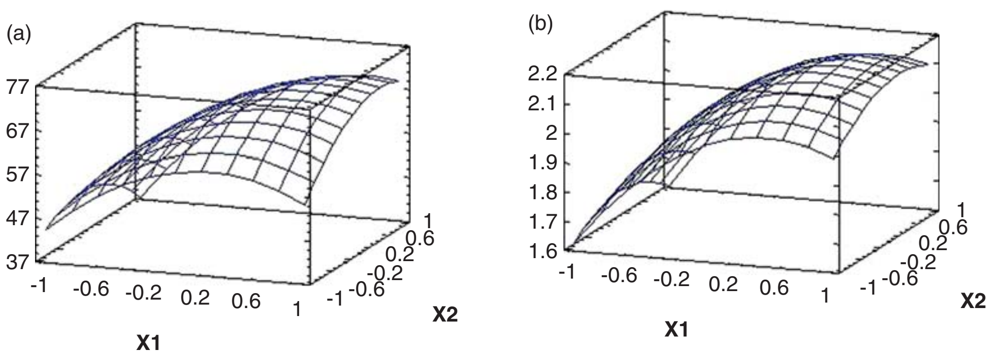

Figure 2. Response surfaces for (a) bread score and (b) specific volume (cm^3/g) as a function of vinal seed flour/corn starch and corn flour/corn starch ratios in specified gluten-free bread formulations.

yeast and 4 g salt (according to the conditions previously mentioned). The average experimental results for the specific volume and the bread score were 2.03 and 67, respectively, whereas the predicted results were 2.09 and 68.77, respectively. Using the hypothesis testing technique, differences between responses from models and from verification experiment were shown not to be significant at the 5% level.

Optimized gluten-free bread characteristics

The optimized gluten-free bread composition is presented in Table V. Presence of gluten was found to be negative in the optimized gluten-free bread. If the 5 ppm gliadin detection limit of the method is taken into account, the product could be considered gluten free as 20 ppm is established as the maximum by Codex Alimentarius (FAO/WHO) (Codex Stan 2008).

Antioxidant activity expressed as mg AEAC from optimized gluten-free bread was 115 ± 20 mg/100 g, meaning that a 100 g portion could give a reduction power similar to a 60 g portion of guava (180 mg ascorbic acid/100g) or 200 ml orange juice (50–60 mg/100ml) (Leong and Tee 2007). Vinal seeds flour should contribute to gluten-free bread antioxidant activity because of its important polyphenol content. Also, Maillard reaction products that result from a condensation reaction between amino acids or proteins and reducing sugars or lipid oxidation products that might occur during baking should be also considered (Mastrocola and Munari 2000; Dewanto et al. 2002).

Figure 3 shows gluten-free bread photographed; as can be seen, corn flour contributes to a better loaf volume and air cells. A bread of light brown color was obtained that was caused in part by its polyphenol presence, which could be an advantage because it contributes to its mentioned antioxidant activity.

Conclusions

An acceptable gluten-free bread, evaluated by an expert panel, was developed containing *P. ruscifolia* (vinal) seeds, which are from an indigenous legume and are under-utilized nowadays, and corn flours. This resulted in a product with higher protein content compared with similar gluten-free breads found in local commercial markets. Also, vinal seed flour contributes to an interesting antioxidant activity in optimized gluten-free bread. These findings contribute to evaluating another utilization for vinal seed flour, which, because of its composition rich in proteins and gums, must be further studied. Also, it must be kept in mind that vinal sustainable exploitation could be important because it grows spontaneously in a low-income rural region.

Table V. Gluten-free bread composition.

Conten	Value
Moisture (g/100 g)	56 ± 1.2
Total protein (g/100 g)	5.2 ± 0.37
Fat (g/100 g)	8.05 ± 0.6
Polyphenols (mg catequine equivalents)	33 ± 8

Data presented as median ± standard deviation, three replications.

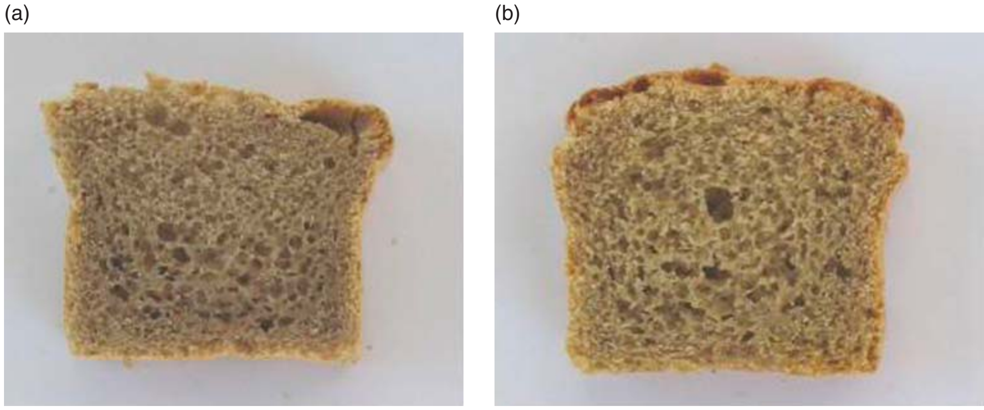

Figure 3. Gluten-free breads formulated (a) with *P. ruscifolia* (vinal) seed flour and (b) with corn and *P. ruscifolia* seed flours.

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