MODIFICATION OF COOKIE-BAKING PROPERTIES OF PEANUT FLOUR BY ENZYMATIC AND CHEMICAL HYDROLYSIS

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ABSTRACT

Defatted peanut (*Arachis hypogaea* L.) flour was suspended in water, adjusted to pH 2.0, 4.5, and 7.6, and enzymatically hydrolyzed with pepsin, bromelain, and trypsin, respectively. Controls received no enzyme treatment. All suspensions were readjusted to pH 6.9, freeze-dried, pulverized, and incorporated in a cookie formula at wheat-flour substitution levels of 5, 15, and 25%. Marked improvement in dough-handling characteristics was achieved through pH and enzyme treatments. Cookies containing untreated or treated peanut flour had generally increased volume and weight. With the exception of the bromelain hydrolysate, the use of peanut flour in cookies resulted in increased specific volume when compared to the 100% wheat-flour control. Untreated peanut flour reduced the diameter and increased the height of cookies. This trend was reversed by hydrolyzing peanut flour protein. Top-grain was better in fortified cookies containing treated peanut flours as compared to those containing untreated material. Treatment of peanut flour resulted in improved appearance. Trypsin hydrolysate, substituted for 25% of wheat flour, produced an undesirable sulfur aroma and eggy flavor in cookies. The bitterness sometimes associated with peptides in hydrolyzed proteins was not detectable in cookie formulations having up to 25% treated peanut flour substitution. Protein and lysine contents of the cookies were increased about 70% by substituting peanut flour for 25% of the wheat flour.

Fortification of wheat flour with high-protein vegetable flours in an attempt to increase the nutritional quality of baked products has been under investigation for many years. The bulk of research effort has been directed toward evaluating the functional properties of soy derivatives in bread (1–6). Performance of wheat-flour components (7), rapeseed isolates and concentrates (8), and peanut flour (5,9) in breads has also been examined. Palatability of pancakes fortified with legume flours has been investigated (10). The use of high-protein cookies as a potential source of improved nutriment for humans has been given less attention because they are not universally consumed (as are breads) and because of the reluctance of some nutritionists to promote snack or dessert items as sources of protein. Nevertheless, interest does exist in fortifying the protein content (11,12) and improving the overall quality of cookies through the addition of flour derivatives (13,14).

Functional properties of dispersed wheat gluten (15) and soy (16) and peanut proteins (17–20) can be greatly modified by enzymatic and chemical hydrolysis. This paper presents data on the cookie-baking characteristics of hydrolyzed peanut proteins.

MATERIALS AND METHODS

Peanut Flour Preparation

Peanut (*Arachis hypogaea* L.) flour was prepared by the following procedure[1]:

[1]J. L. Ayres, Director of Product Development, Gold Kist Research Center, Lithonia, Ga. Personal communication (1975).

runner peanuts were dried, blanched with a Bauer 341 split nut blancher, and electronically sorted to remove darkened kernels. The peanuts were then conditioned with steam, flaked, dried, and extracted with hexane. The defatted peanuts were desolventized by passing them through a tube heated at 104°C. Residence time was 20 min. The extracted, desolventized peanut meal was ground with a Kolloplex mill at 11,200 rpm. The composition and granulation of the flour are as listed in Table I.

Peanut flour was modified by chemical and enzymatic hydrolysis before it was tested in cookie formulations. Forty-five grams of peanut flour was dispersed in 225 ml of distilled water. Slurries were adjusted to pH 2.0 or 4.5 with 6*N* HCl, or to pH 7.6 with 6*N* NaOH. Pepsin (EC 3.4.4.1), bromelain (EC 3.4.4.24), and trypsin type II (Sigma Chemical Co., St. Louis, Mo.) were dissolved in water to yield enzyme:protein ratios of 1:100 upon addition of 30 ml to peanut flour slurries. Pepsin hydrolysis was carried out at pH 2.0, bromelain at pH 4.5, and trypsin at pH 7.6. Slurries were constantly agitated at 50°C for 30 min and then heated at 90°C for 10 min to inactivate the enzymes. Appropriate controls were also prepared and evaluated for cookie-making characteristics. All hydrolysates, including controls, were adjusted to pH 6.9, frozen immediately, freeze-dried, pulverized, and passed through a Wiley mill (60-mesh screen) twice. Table II summarizes peanut flour treatments. Note that Control 1 was the unaltered peanut flour; Controls 2, 3, and 4 represented controls for pepsin, bromelain, and trypsin hydrolysates, respectively.

TABLE I
Composition and Granulation of Peanut Flour

Characteristic	%
Composition	
Moisture	1.2
Protein (N × 5.46)	53.7
Fat (hexane extract)	1.8
Ash	4.8
Fiber	3.9
Carbohydrate[a]	34.6
Granulation	
Through 100 U.S. mesh	100.0
200 U.S. mesh	95.4
325 U.S. mesh	91.2
425 U.S. mesh	65.4

[a]By difference (excludes fiber).

TABLE II
Summary of Peanut Flour Treatments

Sample Designation	pH during 30-Min, 50°C Treatment	Enzyme	10-Min, 90°C Treatment
Control 1	No pH/heat treatment	None	−
Control 2	2.0	None	+
Pepsin hydrolysate	2.0	Pepsin	+
Control 3	4.5	None	+
Bromelain hydrolysate	4.5	Bromelain	+
Control 4	7.6	None	+
Trypsin hydrolysate	7.6	Trypsin	+

Cookie Formulation and Baking

Three levels of peanut flour were substituted for wheat flour. Cookie formulations are presented in Table III. Flour(s), cream of tartar, baking soda, and cinnamon were sifted twice. Eggs were blended for 1 min at speed 1 by a 12-speed Sunbeam Mixmaster, and then creamed with shortening and sugar for 2 min at speed 2, followed by 1 min at speed 6. Dry ingredients were folded into the latter mixture over a 30-sec period at speed 1, and the entire formulation was then mixed for 1 min at speed 3. The batter was rolled to a uniform thickness of 9 mm and cut (30-mm diam). Approximately 3 dozen cookies of each test and control formulation were baked in a preheated oven at 400° F (204° C) for 7.5 min and cooled on a rack.

Physical Characteristics

Weight, diameter, height, and volume (seed displacement method) of cookies were measured 4–6 hr after baking. Data presented represent averages from measurements of 8–10 cookies.

Moisture

Moisture was determined using a Mettler Drying Unit (LP11) attached to a Mettler P120 top-loading balance. The lamp in the drying unit was set at reading No. 5, and 5 g of cookie crumb was exposed to heat for 4 min before weight loss was recorded.

pH Determination

Ten grams of cookie crumb was combined with 30 ml distilled water. A Corning Digital 112 Research pH meter was used to measure pH values of the suspension after 4–5 min of intermittent agitation.

Gardner Color Measurements

Color and lightness of the top surface of cookies were measured using a Gardner Color Difference Meter, Model C-4(L), set against a chromatic reflectance standard (No. SKC-SBC-31, Gardner Laboratory, Inc., Bethesda, Md.). Reference values for the standard were: L = 76.6 (lightness); $a = -1.1$ (green); and $b = 24.3$ (yellow).

TABLE III
Formulation of Cookies Containing Peanut Flour Substituted for Wheat Flour

	Peanut Flour as % of Total Flour			
Ingredient	**0**	**5**	**15**	**25**
Wheat flour (plain, all-purpose)	75.0	71.2	63.7	56.0
Peanut flour	0	3.8	11.3	19.0
Cream of tartar	1.5	...	...	...
Baking soda	1.0	...	...	...
Cinnamon	0.5	...	...	...
Egg (whole)	24.0	...	...	...
Sugar (cane)	75.0	...	...	...
Shortening (hydrogenated vegetable)	47.0	...	...	...

Organoleptic Evaluation

A 10-member sensory panel was instructed to assign scores of 1–9 (1 = extremely poor; 5 = borderline; 9 = excellent) for appearance, color, aroma, texture, and flavor of cookies. Samples were evaluated 4–6 hr after baking. A wheat-flour control cookie (no peanut flour substituted) was included with two test cookies at each panel sitting.

Statistical Analyses

Where appropriate, significance of mean differences in values was determined by Duncan's multiple range test and reported at $P \leqslant 0.01$ level.

RESULTS AND DISCUSSION

Substitution of wheat flour with untreated peanut flour (Control 1) caused a marked stiffening of dough. At the 25% level, dough was crumbly and difficult to handle. Adjustment of peanut flour to pH 2.0, as well as treatment with pepsin at this pH, greatly improved the handling characteristics of dough in which these flours were incorporated. The size of the protein molecule apparently affected dough texture. In an earlier paper (19) we showed that heating peanut flour at pH 2.0, whether in the presence or absence of pepsin, greatly altered proteins qualitatively. Transformation of large-molecular-weight globulins such as arachin to smaller components was demonstrated by polyacrylamide gel electrophoretic techniques. Use of peanut flours treated at pH 4.5, with or without bromelain, and at pH 7.6, with or without trypsin, improved handling properties of cookie dough. These doughs did not tend to crumble and compared favorably in texture to that of the 100% wheat-flour control. The general nature of dough characteristics containing various test flours cannot be correlated with the capacity of these test flours to form emulsions. For example, the emulsion capacity of Control 2 has been shown to be 70% of Control 1, whereas pepsin and trypsin hydrolysate capacities are about 135% (18).

Other functional properties of treated peanut flour may also have been altered, thus affecting dough characteristics. Hermansson *et al.* (21) reported that the swelling ability (ml of water uptake per g) of rapeseed protein concentrate could be reduced by pepsin or acid hydrolysis. The sugar:flour ratio (22) and the extent to which peanuts and peanut paste are heated (23–25) also greatly affect functional performance. In the latter study, cookie batters prepared from peanuts heated for longer times were consistently smoother, thinner, and required less shaping than those heated for shorter times. The 50°C/30 min and 90°C/10 min treatments administered to flours in this study may have contributed to the less crumbly nature of doughs in which these flours were incorporated.

Some physical and chemical characteristics of test and control cookies are listed in Table IV. Volume, at least at the 25% substitution level, was increased as compared to the 100% wheat-flour control. Substitution of wheat flour with either treated or untreated peanut flour in formulations generally resulted in higher weight per cookie. With the exception of the bromelain hydrolysate, the specific volume of cookies was increased through the addition of peanut flour. Therefore, the density of cookies containing peanut flour was generally less than that of the 100% wheat-flour control.

TABLE IV
Physical and Chemical Characteristics of Cookies Containing Various Levels of Peanut Flour

Flour	Flour Substitution[a] %	Volume cc	Weight g	Specific Volume v/wt	Diameter[b] mm	Height[b] mm	Spread Ratio diam/ht	Moisture %	pH
Wheat	0	15.0	6.74	2.23	46.4efg	4.2bcd	11.0	4.6	6.41
Control 1	5	15.8	6.78	2.33	46.0efgh	4.3cd	10.7	4.1	6.38
	15	17.5	7.16	2.43	46.3efgh	4.2cd	11.0	4.7	6.47
	25	20.1	7.54	2.67	43.7h	4.9a	8.9	4.9	6.61
Control 2	5	16.3	6.78	2.40	46.7defg	4.2bcd	11.1	4.3	6.45
	15	16.2	6.83	2.37	46.7defg	4.2bcd	11.1	5.0	6.48
	25	16.8	6.87	2.45	49.3bcd	3.9de	12.6	4.2	6.59
Pepsin hydrolysate	5	15.8	6.52	2.42	52.3a	4.1cd	12.8	3.6	6.39
	15	15.9	6.74	2.36	47.0cdefg	4.2bcd	11.2	4.2	6.47
	25	18.6	6.90	2.69	49.3bcd	4.1cd	12.0	4.0	6.72
Control 3	5	16.1	6.70	2.40	47.7bcdefg	4.2bcd	11.4	4.6	6.45
	15	16.5	6.95	2.37	50.0ab	3.7ef	13.5	4.7	6.62
	25	17.7	7.26	2.44	47.7bcdefg	3.9de	12.2	4.0	6.65
Bromelain hydrolysate	5	14.9	6.71	2.22	45.7fgh	4.2bcd	10.9	5.3	6.54
	15	14.0	6.83	2.05	47.3bcdefg	3.9de	12.1	4.4	6.62
	25	15.3	7.21	2.12	50.0ab	3.9de	12.8	4.3	6.74
Control 4	5	16.4	6.81	2.41	47.7bcdefg	4.1cd	11.6	4.6	6.60
	15	17.8	7.13	2.50	48.7bcde	4.1cd	11.9	3.9	6.63
	25	18.9	7.07	2.67	48.0bcdef	3.7ef	13.0	3.6	6.72
Trypsin hydrolysate	5	16.3	6.88	2.37	49.7abc	3.7ef	13.4	4.0	6.50
	15	16.3	7.20	2.26	49.7abc	3.5f	14.2	4.5	6.60
	25	19.7	7.21	2.73	49.3bcd	4.1cd	12.0	4.6	6.67

[a]Percentage of wheat flour substituted with peanut flour.
[b]Values followed by the same letter do not differ significantly at $P \leqslant 0.01$.

Untreated peanut flour (Control 1) substitution reduced the diameter and increased the height of cookies. These changes were significant at the 25% substitution level. The use of cottonseed flour in a similar cookie formulation has also been noted to decrease the mean width and height (12). Laboratory-prepared wheat gluten and soy flour derivatives have also been reported to reduce cookie spread rapidly with increasing concentration and degree of functionality (11). Treatment of peanut flour with proteolytic enzymes reversed their behavior with respect to controlling cookie spread. As evidenced by substantial increases in spread ratios, the diameter of cookies containing treated flours increased proportionately more than did the height. These data promote the feasibility of decreasing or increasing the spread of cookies through the addition of various amounts of untreated or enzyme-hydrolyzed peanut flour.

The moisture content of test and control cookies was similar. In an earlier paper (19), we reported that enzymatically hydrolyzed peanut flour could adsorb more water than the untreated control at specific equilibrium relative humidities. Apparently the retention of water by peanut flours was unaffected by hydrolysis

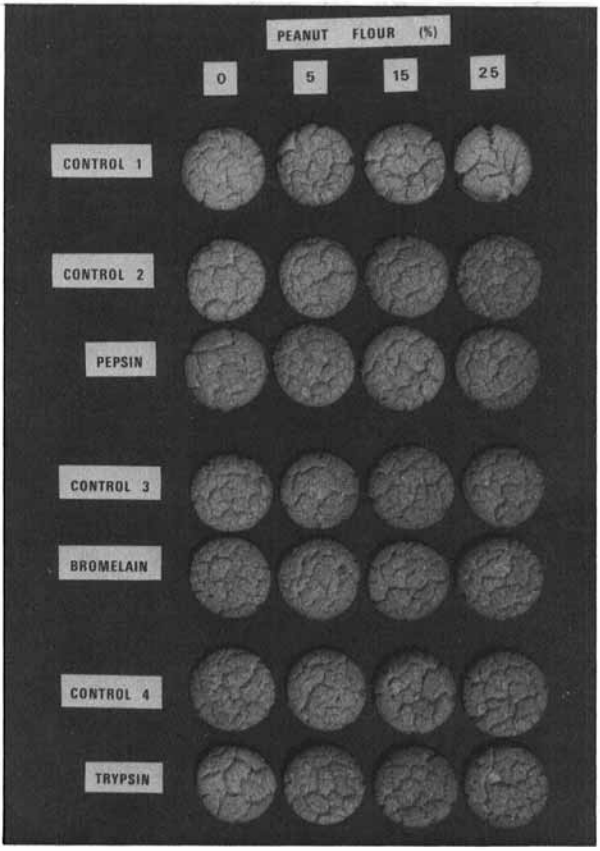

Fig. 1. Representative cookies containing peanut flour substituted for wheat flour.

when evaluated in the cookie test system.

The use of peanut flour in place of wheat flour tended to cause an increase in pH of cookie crumb. The change was not substantial.

Figure 1 shows representative cookies containing peanut flour substituted for wheat flour at 0, 5, 15, and 25% levels. Top-grain was adversely affected by incorporating untreated peanut flour (Control 1) at 5% or higher. Restoration of top-grain appearance could be achieved through pH adjustment of peanut flour, with or without treatment with proteolytic enzymes. Cookies containing 25% untreated peanut flour were undesirably light in color. Color-producing characteristics were induced by treating the peanut flour with acid, base, and enzymes, either singly or in combination.

Objective color data for top surfaces of cookies are summarized in Table V.

TABLE V
Objective Color and Lightness Values for Top Surfaces of Cookies Containing Various Levels of Peanut Flour

Flour	Flour Substitution[b] %	Gardner Value[a] L	Gardner Value[a] $-a$	Gardner Value[a] $+b$	Color Difference E[c]
Wheat	0	58.2bcdef	0.2	17.9bc	19.6
Control 1	5	57.9bcdef	0.3	17.7bc	19.7
	15	57.5bcdefgh	0.1	17.2bc	20.4
	25	62.8a	0	21.8a	14.1
Control 2	5	57.6bcdefgh	0.1	17.3bc	20.2
	15	55.1defghij	0.4	16.7bc	22.7
	25	54.1ghij	0	16.4bc	23.9
Pepsin hydrolysate	5	56.1cdefghij	0	16.2c	22.0
	15	53.8hij	0	16.0c	24.3
	25	54.9efghij	0.3	17.4bc	22.9
Control 3	5	57.6bcdefg	0.9	17.0bc	20.3
	15	52.9j	0	16.7bc	24.9
	25	57.8bcdefg	0.1	16.9bc	20.4
Bromelain hydrolysate	5	59.0bcd	0	16.9bc	19.1
	15	55.4defghij	0.1	16.4bc	22.7
	25	57.1bcdefghi	0	17.8bc	20.5
Control 4	5	59.4abc	0.3	17.5bc	18.5
	15	54.9efghij	0.2	17.3bc	22.9
	25	53.6ij	0	16.3c	24.3
Trypsin hydrolysate	5	55.1efghij	0	17.3bc	22.6
	15	53.6ij	0.3	17.5bc	24.0
	25	56.3bcdefghij	0.1	21.5a	20.5

[a]Values followed by the same letter do not differ significantly at $P \leqslant 0.01$.
[b]Percentage of wheat flour substituted with peanut flour.
[c]$E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$, where ΔL, Δa, and Δb are respective differences between standard and sample.

Lightness (L) of cookies was significantly higher when untreated peanut flour was substituted for wheat flour at 25%. The effects of various levels of treated flours are not entirely clear; however, significant darkening was observed as a result of some pH and enzyme treatments. It should be noted that darkening was generally in the form of brownish or golden color changes and not green or gray as sometimes associated with vegetable flours. Overall, there were no dramatic color differences (E) in cookies prepared from wheat and peanut flours.

Sensory evaluation scores for cookies are presented in Table VI. The undesirable appearance of cookies containing 25% untreated peanut flours (Control 1) was attributed to pale coloration and poor top-grain. The trypsin hydrolysate, when substituted for wheat flour at the 25% level, resulted in a sulfur aroma and cookies produced from it were described as "eggy." The bitterness associated with pastes prepared from pepsin-hydrolyzed peanut flour (18) was not detected in cookies containing up to 25% of the product as a flour

TABLE VI
Sensory Evaluation Scores for Cookies Containing Various Levels of Peanut Flour

Flour	Flour Substitution[b] %	Sensory Score[a]				
		Appearance	Color	Aroma	Texture	Flavor
Wheat	0	7.8ab	7.6a	7.7ab	7.4a	7.5ab
Control 1	5	7.6ab	7.9a	7.3abc	7.5a	7.6ab
	15	7.8ab	7.7a	7.1abcd	6.9a	7.1ab
	25	4.8c	6.0b	6.2bcd	6.3a	6.5ab
Control 2	5	8.1a	8.2a	7.5ab	7.4a	7.1ab
	15	7.7ab	7.7a	7.2abcd	7.0a	6.6ab
	25	6.7b	6.9ab	5.9cd	6.2a	6.5ab
Pepsin hydrolysate	5	7.8ab	7.6a	7.2abcd	7.5a	7.1ab
	15	7.9ab	7.5a	6.7abcd	7.6a	7.0ab
	25	7.4ab	7.7a	6.9abcd	6.3a	6.0b
Control 3	5	7.6ab	7.4a	7.4abc	7.6a	7.6ab
	15	7.7ab	7.6a	7.1abcd	7.3a	7.0ab
	25	7.6ab	7.2a	7.3abc	6.4a	7.0ab
Bromelain hydrolysate	5	7.9ab	7.9a	7.1abcd	7.2a	7.3ab
	15	8.0a	7.9a	7.2abcd	7.1a	6.9ab
	25	7.6ab	7.8a	6.2bcd	7.0a	6.5ab
Control 4	5	7.6ab	7.4a	7.5ab	7.5a	7.8a
	15	7.9ab	7.9a	7.8a	7.5a	7.7ab
	25	7.4ab	7.2a	7.7ab	6.9a	6.6ab
Trypsin hydrolysate	5	7.6ab	7.6a	7.7ab	7.6a	7.8a
	15	7.6ab	7.8a	7.6ab	7.4a	7.4ab
	25	7.6ab	7.9a	5.7d	6.2a	3.9c

[a]Scored on a scale of 1–9, where 1 = extremely poor, 5 = borderline, and 9 = excellent. Values within a column followed by the same letter do not differ significantly at $P \leq 0.01$.
[b]Percentage of wheat flour substituted with peanut flour.

component. Although untreated peanut flour was less compatible than treated flours with respect to dough-mixing properties and the ability to maintain appearance and color of cookies, it was judged no less acceptable in flavor.

Having demonstrated the improvement of certain cookie-baking properties of peanut flour through enzymatic hydrolysis, the question that must be asked is whether there is any improvement in the nutritional value of products such as cookies in which the hydrolysates might be incorporated. Assuming the finished cookie contained 4.5% moisture, on a dry weight basis, the following percentages of protein (wheat = N × 5.7; peanut = N × 5.46) can be calculated from the product formulation[2]:

Peanut Flour Substitution, %	*Total Protein, %*
0	5.6
5	6.3
15	7.9
25	9.5

The quantity of protein contained in a given weight of cookie is increased about 70% by substituting peanut flour for 25% of the wheat flour. Lysine, which is the most limiting amino acid in the wheat-flour cookie, is increased in proportion to the increase in protein with increments of peanut flour substitution. Nutritional quality can be determined, however, only through animal-feeding studies. In this way, the biological availability of the products of hydrolysis in the peanut flour and in the finished cookie can be evaluated.

[2]Whole raw egg contains 3.07% protein, 74% water; wheat flour contains 10.2% protein.

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