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Amylose-lipid complex formation during cooking of rice flour

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Abstract

The effects of cooking rice flour in the presence of myristic, palmitic and stearic acid on amylose-lipid complex formation, water solubility and pasting properties were studied. Different fatty acids were added to rice flour, at 1.5, 3.0 and 4.5% levels, and cooked in Visco-amylograph at 95°C for 30, 60 and 90 min. Amylose-lipid complex formation increased and water-solubility decreased with the increase in levels of all the fatty acids in rice paste cooked for 30–90 min. Complexing of amylose with all the fatty acids increased with the increase in cooking time. Myristic acid had the highest ability to form the complex and stearic acid the lowest. Iodine spectra of rice paste cooked with and without lipids were also determined to confirm the formation of amylose-lipid complexes. Both λ_{max} and ratios of absorbances at 630 and 520 nm decreased with the increase in levels of all the fatty acids. Myristic acid caused a greater reduction in λ_{max} and ratios of absorbance. Addition of all the fatty acids increased the pasting temperature, peak viscosity, viscosity at 95°C and viscosity at 50°C of rice paste. Consistency coefficient and flow behaviour indices of rice pastes cooked in the presence of different fatty acids were also determined using a Brookfield viscometer. Consistency coefficient increased with the increase in levels of all the fatty acids and the increase was more pronounced with myristic acid. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Rice; Myristic acid; Palmitic acid; Stearic acid; Complexing index; Pasting properties

1. Introduction

Additions of fatty acids alter the physical and chemical properties of starchy foods (Singh, Cairns, Morris & Smith, 1998). The changes brought about by them in starchy foods have been attributed to the formation of complexes between amylose and lipids. Lipids have been reported to form inclusion compounds with amylose, with the hydrocarbon portion of the lipid located within the helical cavity of amylose (Banks & Greenwood, 1972). The complex has been reported to decrease water-solubility and susceptibility of the starches to alpha amylase digestion (Eliasson & Krog, 1985; Seneviratne & Biliaderis, 1991).

Hoover and Hadziyev (1981) used swelling power and solubility tests to show that myristic acid was the most effective fatty acid in forming complexes with starches. Lagendijk and Pennigs (1970) found that complexing index decreased as the number of double bonds increased. Mercier, Charbonniere, Grebaut and De La Gueriviere (1980) showed, using twin-screw extrusion, that values for water-soluble carbohydrates and retrogradation decreased when oleic acid or linoleic acid were used in place of stearic acid. Hahn and Hood (1987) reported that increasing unsaturation resulted into less binding. Nierle and El Baya (1990) studied the effect of different lipids on viscosity and pasting properties of wheat starch. Hibi (1994) reported the effect of palmitic acid and emulsifiers on viscoelastic properties of rice starch gel.

The present study was undertaken to examine the effects of cooking of rice flour in the presence of palmitic, myristc and stearic acid (99% purity) on complexing index, iodine spectra, solubility, pasting and rheological properties.

2. Materials and methods

2.1. Material

A paddy sample of variety PR-106 was procured from the 1997 harvest. Palmitic acid, myristic acid and stearic acid were obtained from Sigma (Poole, UK).

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2.2. Preparation of samples

Clean paddy was dehusked and polished to a uniform 6% degree of polish as described earlier (Singh, Sekhon & Kaur, 1990). Milled rice was obtained and ground in a Brabender Quadrumat Junior Mill.

2.3. Pasting and cooking of rice paste

Pasting properties of rice with and without lipids were studied using a Brabender Visco-amylograph equipped with 350 cm-g sensitive cartridge and cooling coil. A 40 g sample (14%, moisture basis) was blended with 450 ml of distilled water to obtained a smooth dispersion. The dispersion was heated from 30°C to 95°C (1.5°C/min) and held at 95°C for 30, 60 and 90 min and then cooled to 50°C (1.5C/min). Different fatty acids (palmitic, myristic and stearic acid) were also added at 1.5, 3.0 and 4.5% levels (on flour weight basis) to study their effects on pasting properties. The cooked rice samples were also analysed for complexing index, water-solubility, iodine spectra and rheological properties.

2.4. Complexing index

Complexing index (CI) was determined using the method of Gilbert and Spragg (1964). This method involves the formation of starch-iodine complex and measurement of starch which is not complexed with lipids. The absorbance is related to the portion of starch that is complexed to the iodine. The iodine solution used for analysis was prepared by dissolving 2 g of potassium iodide and 1.3 g of I_2 in 50 ml of distilled water and allowing it to dissolve for about 2 h. Then the final volume was made to 100 ml using distilled water. A cooked rice paste sample (5 g) was mixed with 25 ml of distilled water in a test tube. The test tube was vortexed for 2 min and centrifuged for 15 min at 3000 rpm. The supernatant (500 μl) and distilled water were added to iodine solution (2 ml). The tube was inverted several times and the absorbance was measured at 690 nm. CI was calculated from the equation.

$$\text{CI}(\%) = (\text{Absorbance of control} - \text{absorbance of sample}) \times 100/\text{absorbance of control}.$$

2.5. Water-solubility

For determination of water-solubility of starch, 2.5 g of cooked rice paste was dispersed with 25 ml of distilled water in a beaker. After stirring for 30 min, the dispersions were transferred into a centrifuge tube and the beakers were rinsed with 10 ml of water. The samples were centrifuged at 3000 rpm for 15 min. The supernatant was decanted and determined for solids contents using the following equation.

$$\text{Water solubility index} = \text{weight of dissolved solids in supernatant} \times 100/\text{weight of sample}$$

2.6. Iodine spectra of cooked paste

The method of Sowbhagya and Bhattacharya (1971) was used to determine the iodine spectra of cooked rice pastes. The absorbance spectra of rice pastes cooked with and without lipids prepared as described above were measured using a UV-visible spectrophotometer (UV-1601, Shimadzu) from 400–700 nm and wavelengths of maximum absorption. The ratios of absorbances at 630 and 520 nm for rice flour pastes cooked with and without lipids were also determined (Bhatnagar & Hanna, 1994).

2.7. Rheological properties

Rice pastes cooked in a Brabender visco-amylograph with and without lipids at 95°C for 30, 60 and 90 min were also studied for their flow properties. A Brookfield viscometer (Model DV-II, Brookfield Engineering Labs, Inc., Stoughton, MA) was used to measure consistency coefficient (k) and flow behaviour indices (n) of cooked rice pastes as described earlier for honey (Singh & Bath, 1997). The cooked pastes were transferred in a 250 ml beaker and brought to a temperature of 30°C in a TC-500 water bath (Brookfield Engineering Inc.). Measurements were taken 2 min after the spindle (No.18) was immersed, so as to allow a thermal equilibrium in the sample and to eliminate the effect of immediate time-dependency.

2.8. Statistical analysis

The analysis was carried out in duplicate and the data obtained were subjected to analysis of variance (ANOVA) using Minitab statistical software (Minitab Inc., USA).

3. Results and discussion

3.1. The effects of fatty acids on complexing indices

Mean square errors from the analysis of variance (ANOVA) of complexing indices of rice paste cooked in the presence of myristic, palmitic and stearic acid are shown in Tables 1 and 2. Both the levels of fatty acids

Table 1
Effect of fatty acid levels and cooking duration on complexing index

Cooking duration (min)	Levels (%)	Myristic acid	Palmitic acid	Stearic acid
30	0	–	–	–
30	1.5	18.25	15.95	13.60
30	3.0	20.30	18.80	16.90
30	4.5	26.15	23.55	21.5
60	0	–	–	–
60	1.5	21.50	17.85	15.20
60	3.0	24.60	19.25	17.90
60	4.5	27.50	23.70	22.50
90	0	–	–	–
90	1.5	25.60	19.50	18.90
90	3.0	27.60	21.50	20.80
90	4.5	30.80	27.80	24.30

Table 2
Analysis of variance for complexing indices

Source of variance	df	Mean squares error		
		Myristic acid	Palmitic acid	Stearic acid
Fatty acid levels	2	62.8***[a]	82.9***	73.18***
Cooking duration	2	62.1***	20.3***	25.0***
Fatty acids levels × cooking duration	4	1.72***	1.02***	0.875***
Error	9	0.001	0.000	0.000
Total	17			

[a] *** $P < 0.005$.

and cooking duration showed significant effects on the complexing index. Fatty acid levels and cooking duration interaction effects on the complexing index were also significant. The complexing index progressively increased with the increase in levels of fatty acids and cooking time; however, the acid levels showed the more pronounced effect. Significant differences in ability to complex formation were observed among the fatty acids. Myristic acid showed the highest complexing ability, followed by palmitic and stearic acid. For myristic acid, the complexing indices after 30, 60, 90 min of cooking, at 1.5% addition level were 18.2, 21.5 and 25.6%, respectively, while for stearic acid they were 13.5, 15.2% and 18.8%. An increase in complexing indices with the increase in fatty acids clearly indicates the formation of amylose-lipid complexes.

3.2. The effect of fatty acids on λ_{max}

The λ_{max} values for rice flour pastes cooked with and without fatty acids was determined to confirm the formation of starch-lipid complexes (Table 3). Mean square errors from ANOVA of λ_{max} for rice pastes cooked with different fatty acids are shown in Table 4. Cooked rice pastes showed λ_{max} at 569–576 nm. The λ_{max} values of cooked rice pastes decreased with the increase in levels of all fatty acids and cooking duration, however, the effect of fatty acid levels was more pronounced. The statistical analysis revealed a greater decrease in λ_{max} with myristic acid than palmitic acid or stearic acid, confirming higher complexing ability of myristic acid. Sokhey and Chinnaswamy (1993) reported the absorbance maxima to be 520 nm for amylopectin, branched or short chain fractions and 630 nm for amylose, linear or long-branched chain fractions. Bhatnagar and Hanna (1994) observed λ_{max} to be 520 nm for native waxy starches and 594–603 for native starches with amylose contents in the range 25–70%. They also observed a shift in λ_{max} towards the amylopectin side in native starch extruded with myristic and stearic acid. The decrease in λ_{max} indicated a reduction in availability of amylose in aqueous solution to form iodine-amylose complexes. The decrease in λ_{max} with increase in cooking duration of rice pastes cooked with and without lipids may be attributed to degradation of the linear fraction, to amylose-lipid complex formation or to both of these (Sokhey and Chinnaswamy). The changes in λ_{max} with extended cooking duration may be attributed to an increase in degradation of amylose macromolecules due to the shearing action of pins in the visco-amylograph bowl; hence the chains of amylose may not be long enough to form iodine-amylose complexes during analysis (Fan, Singh & Pinthus, 1997).

3.3. The effect of fatty acids on ratio of absorbances

The change in the ratio of absorbances of iodine-polysaccharide complexes at 630 and 520 nm has been reported to be an indicator of a change in the composition of linear or branched fraction of starch molecules (Bhatnagar & Hanna, 1994; Sokhey & Chinnaswamy, 1992). The 630/520 nm ratio of rice paste decreased with increase in the levels of fatty acids and cooking time (Table 3). Statistical analysis in Table 4 showed that the myristic acid caused greater reduction in absorbance at 630/520 nm than palmitic or stearic acid. During heating of starch and water, amylose leaches out of starch granules and, in the presence of lipids, leaching of soluble carbohydrates in the supernatant decreased leading to the reduction in ratios at 630/520 nm and λ_{max}. The decrease in ratios at 630/520 nm may be attributed to the same factors which are responsible for a decrease in λ_{max} as described above.

3.4. The effect of fatty acids on water solubility indices

The statistical analysis showed the significant effect of both fatty acid levels and cooking duration. The fatty acid level and cooking duration interaction effect on WSI was also observed to be significant (Table 4).

Table 3
Effect of fatty acids levels and cooking time on λ_{max}, aborbance ratio (630/520nm) and WSI

Cooking duration (min)	Levels (%)	Myristic acid			Palmitic acid			Stearic acid		
		λ_{max}	Ratio (630/520 nm)	WSI	λ_{max}	Ratio (630/520 nm)	WSI	λ_{max}	Ratio (630/520nm)	WSI
30	0	576	0.9956	28.4	576	0.9956	28.4	576	0.9956	28.4
30	1.5	574	0.9127	19.7	575	0.9520	24.7	575	0.9877	26.4
30	3.0	566	0.9042	17.6	570	0.9317	21.2	573	0.9498	22.6
30	4.5	545	0.8826	16.6	560	0.9113	19.2	567	0.9275	20.6
60	0	574	0.9856	27.85	573	0.9856	27.8	573	0.9856	27.8
60	1.5	571	0.9092	19.25	571	0.9356	22.1	572	0.9706	23.6
60	3.0	565	0.8955	16.30	566	0.9158	17.4	567	0.9216	20.6
60	4.5	540	0.8730	14.50	564	0.8836	15.7	565	0.9017	19.0
90	0	570	0.9706	26.50	571	0.9706	26.4	569	0.9706	26.4
90	1.5	564	0.8646	16.60	567	0.9128	20.9	568	0.9527	20.9
90	3.0	560	0.8576	15.00	562	0.8826	19.3	565	0.9017	19.3
90	4.5	540	0.7528	13.70	561	0.8613	15.9	564	0.8921	15.9

Table 4
Analysis of variance for λ_{max}, ratio (630/520nm) and WSI

Source of variance	df	Mean squares error								
		Myristic acid			Palmitic acid			Stearic acid		
		λ_{max}	Ratio (630/520 nm)	WSI	λ_{max}	Ratio (630/520 nm)	WSI	λ_{max}	Ratio (630/520 nm)	WSI
Fatty acid levels	3	1197***[a]	0.02272***	195.4***	141.7***	0.01034***	142.9***	66.9***	0.008058***	89.1***
Cooking duration	2	92.2***	0.00923***	14.5***	76.2***	0.003552***	36.05***	78.5***	0.002551***	31.0***
Fatty acids levels×cooking duration	6	4.4***	0.0014***	1.09***	13.3***	0.00009***	1.59***	3.38***	0.000061***	1.43***
Error	12	2	0	0.048	2	0.000005	0.022	2	0.000002	0.029
Total	23									

[a] $***P < 0.005$.

Water-solubility progressively decreased with the increase in level of fatty acids. However, the decrease was more pronounced with the addition of myristic acid (Table 3). Similar effects of addition of palmitic acid to the rice starch on water-soluble carbohydrates have been reported earlier (Hibi et al., 1994). The decrease in water-solubility with the addition of myristic, palmitic and stearic acid corroborate the complexing index results. A decrease in solubility index of wheat starch extruded in the presence of myristic, palmitic and stearic acid has been observed earlier (Singh et al., 1998).

3.5. The effect of fatty acids on pasting properties

Pasting temperature, peak viscosity, viscosity at 95°C and viscosity at 50°C of rice flour increased with the increase in levels of fatty acids (Table 5). Statistical analysis showed a significant effect of all the fatty acids on all pasting properties (Table 6). The changes in viscosity at 50°C were more pronounced with myristic acid than with stearic or palmitic acid. Stearic acid showed a greater effect on pasting temperature and peak viscosity. The increase in pasting temperature, with addition of fatty acids, may be attributed to amylose-lipid complex formation. Goering, Jackson and Dehaas (1975) reported that lipid removal gave a somewhat reduced pasting temperature, negligible pasting peak, improved cooking stability and reduced set-back. Melvin (1979) reported that lipid removal from corn and wheat starches reduced the pasting temperature but increased the pasting peak, paste consistency and set-back. Amylose complexing agents have been reported to increase pasting temperature, paste consistency and set-back viscosity with wheat and corn starch (Krog, 1973) but to lesser extent with tapioca (Moorthy, 1985). Takahashi and Seib (1988) also observed an increase in pasting peak and consistency when prime wheat starch was impregnated with 2% wheat lipids. The change in peak viscosity, viscosity at 95 and 50°C with the addition of fatty acids, may be attributed to the formation of amylose-lipid complexes.

3.6. The effects of fatty acids on flow behaviour

The consistency coefficient (k) of cooked rice pastes increased with the increase in fatty acids levels and

Table 5
Effect of fatty acids levels and cooking duration on pasting properties of rice flour

Cooking duration (min)	Levels (%)	Myristic acid				Palmitic acid				Stearic acid			
		PT[a]	PV[b]	Viscosity at 95°C	Viscosity at 50°C	PT	PV	Viscosity at 95°C	Viscosity at 50°C	PT	PV	Viscosity at 95°C	Viscosity at 50°C
30	0	74.5	430	385	1175	74.5	430	385	1175	74.5	430	385	1175
30	1.5	78.2	480	475	1350	74.5	500	460	1260	82.5	520	460	1200
30	3.0	80.2	500	500	1400	78.2	520	485	1285	82.6	545	490	1260
30	4.5	82.5	530	520	1430	80.2	550	500	1310	83.0	565	510	1285
60	0	–	–	–	1205	–	–	–	1205	–	–	–	1205
60	1.5	–	–	–	1420	–	–	–	1280	–	–	–	1260
60	3.0	–	–	–	1450	–	–	–	1300	–	–	–	1290
60	4.5	–	–	–	1470	–	–	–	1330	–	–	–	1310
90	0	–	–	–	1250	–	–	–	1250	–	–	–	1250
90	1.5	–	–	–	1445	–	–	–	1310	–	–	–	1280
90	3.0	–	–	–	1470	–	–	–	1330	–	–	–	1310
90	4.5	–	–	–	1500	–	–	–	1365	–	–	–	1340

[a] PT, Pasting temperature.
[b] PV, Peak viscosity.

Table 6
Analysis of variance for pasting characteristics

Source of variance	df	Mean squares error											
		Myristic acid				Palmitic acid				Stearic acid			
		PT[a]	PV[b]	Viscosity at 95°C	Viscosity at 50°C	PT	PV	Viscosity at 95°C	Viscosity at 50°C	PT	PV	Viscosity at 95°C	Viscosity at 50°C
Fatty acid levels	3	69**[c]	10289**	20239**	81924**	72.6**	14928**	16623**	16964**	94**	19634**	25706**	11668**
Cooking duration	2	0	4.2	16.7	11601**	0	11.5	9.4	6584**	0	29*	1837*	8332**
Fatty acids levels× cooking duration	6	0	26.4	22.2	158*	0	22	23.3	77.4	0	9,7	460	196*
Error	12	0.02	28.1	20.8	56	0.2	12.3	11.5	39.6	0	5	312	37.5
Total	23												

[a] PT, Pasting temperature;
[b] PV, Peak viscosity
[c] $*P<0.05$, $**P<0.005$.

cooking duration (Tables 7 and 8). The addition of myristic acid caused a greater increase in k value than palmitic acid or stearic acid. The k values of rice pastes cooked for 30 min at 0, 1.5, 3.0 and 4.5% addition levels of myristic acid were 10.9, 13.1, 13.9 and 14.4 Pa s^n, respectively, while the k values for palmitic acid were 10.9, 12.6, 13.6 and 13.8Pa s^n, and, for stearic acid, were 10.9, 12.3, 12.5 and 13.7 Pa s^n, respectively, at corresponding addition levels. The variation in k values caused by different fatty acids appears to be due to the differences in their ability to form complexes with amylose. Flow behaviour indices (n) of cooked rice pastes were less than 1. Therefore, the fluids can be characterized as pseudoplastic. The addition of fatty acids decreased n values. The k value of 1.2–2.4 Pa s^n and n values of 0.2–0.5 for non-waxy rice starch-emulsifier solution has been reported earlier (Guraya, Kadam & Champagne, 1997). The increase in k value with the

Table 7
Effect of fatty acids levels and cooking duration on flow behaviour

Cooking duration (min)	Levels (%)	Myristic acid		Palmitic acid		Stearic acid	
		k (Pa s^n)	n	k (Pa s^n)	n	k (Pa s^n)	n
30	0	10.9	0.362	10.9	0.362	10.9	0.362
30	1.5	13.1	0.285	12.6	0.296	12.3	0.336
30	3.0	13.9	0.276	13.2	0.279	12.5	0.306
30	4.5	14.4	0.274	13.8	0.277	13.6	0.297
60	0	11.1	0.303	11.1	0.303	11.1	0.303
60	1.5	14.1	0.262	13.3	0.268	12.6	0.295
60	3.0	14.5	0.216	14.2	0.260	13.2	0.285
60	4.5	16.6	0.158	14.3	0.254	13.9	0.277
90	0	12.6	0.297	12.6	0.297	12.6	0.297
90	1.5	14.8	0.214	14.2	0.255	13.5	0.262
90	3.0	16.6	0.158	14.3	0.196	14.1	0.205
90	4.5	17.8	0.156	16.6	0.158	14.2	0.198

Table 8
Analysis of variance for k and n

Source of variance	df	Mean squares error					
		Myristic acid		Palmitic acid		Stearic acid	
		k	n	k	n	k	n
Fatty acid	3	25.30**[a]	0.017**	12.70**	0.009**	6.529**	0.005*
Cooking time	2	11.8**	0.018**	7.2**	0.011**	3.75**	0.014**
Fatty acid× cooking time	6	0.556**	0.001**	0.493**	0.001**	0.176**	0.0006*
Error	12	0.015	0.000	0.0175	0.000	0.0162	0.0002
Total	23						

[a] $**P<0.005$, $*P<0.05$

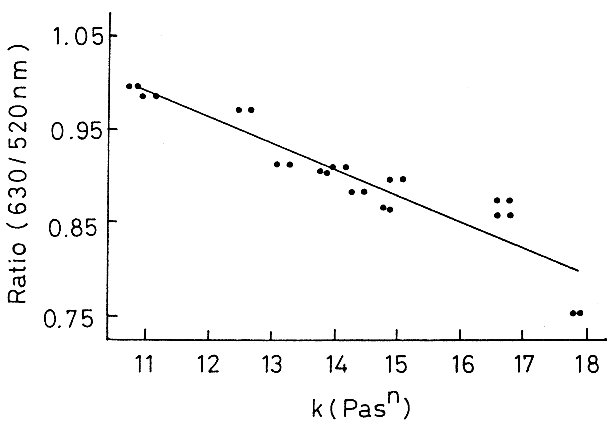

Fig. 1. The relationship between k and ratios at (630/520 nm) for rice paste cooked in the presence of myristic acid.

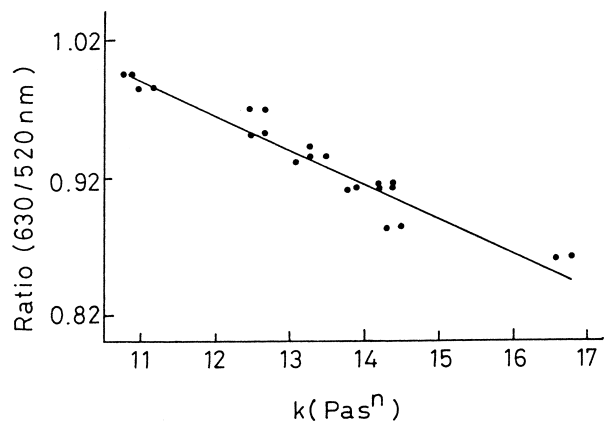

Fig. 2. The relationship between k and ratios at (630/520 nm) for rice paste cooked in the presence of palmitic acid.

addition of fatty acids may be attributed to formation of inclusion complexes with starch. The changes in k values with different fatty acids depend on their ability to form complex e.g. myristic acid showed the highest complexing ability and hence showed greater change in k value. The relationship between k values and ratios of absorbance of iodine-starch at 630/520 nm, measured for rice pastes cooked with and without fatty acids, showed R^2 values of 0.8, 0.86 and 0.9 for myristic acid, palmitic acid and stearic acid, respectively, which also revealed that complex formation affects rheological properties of pastes (Figs. 1–3). The changes in k values correlate well with the viscosity at 50°C measured using a Visco-amylograph. Raphaelides (1992) also observed an increase in viscosity of aqueous amylose and amylose/amylopectin solutions at pH 12 with the addition of palmitic, stearic, oleic and arachidic acids at sufficiently high concentration.

Addition of fatty acids significantly affected the complexing indices, λ_{max}, ratios of absorbances at 630/520 nm, water-solubility, flow behaviour indices and pasting properties of rice flour. Myristic acid was observed to be more effective in altering these properties than palmitic or stearic acid. The rheological properties of cooked rice paste were observed to be significantly affected by fatty acid types and to depend on ability of a particular fatty acid to complex.

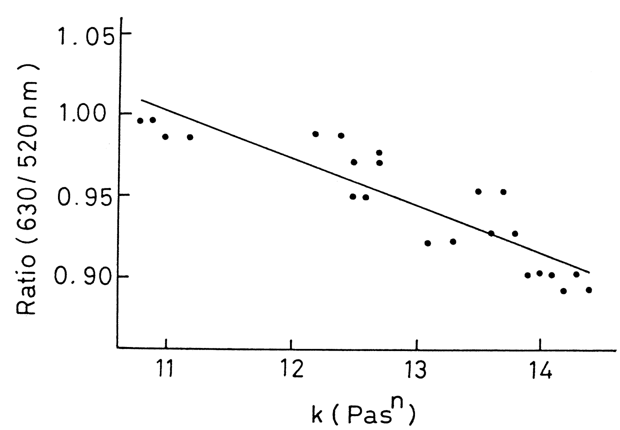

Fig. 3. The relationship between k and ratios at (630/520 nm) for rice paste cooked in the presence of stearic acid.

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