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Effect of amylose on starch pastes viscoelasticity and cooked grains stickiness in rice from seven argentine genotypes

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Abstract

The content of soluble amylose (SAM) in starches from seven novel argentine genotypes was measured. Viscolelastic properties (G'_{max}, G', G', tg θ y η^*) in systems with different starch concentrations (5%, 10%, 15% and 20% w/w) were evaluated by dynamic rheometry. Elasticity dependence upon concentration followed the power law and increased with amylose content. The SAM content explained the differences in elasticity between genotypes of similar total amylose (TAM) content. It was found that a minimum TAM and SAM should be required in order to reduce stickiness in cooked grains.

Keywords: Rice; Starch; Amylose; Rheology; Stickiness

1. Introduction

Rice is a cereal mainly consumed as a whole grain. It is composed about 90% (dry base) of starch and gelatinization features strongly influence cooked rice texture (Juliano, 1985).

Starch consists of two polydispersed α-D-glucan components, amylose (AM) and amylopectin (AP). The AM is linear (α-D-[1 → 4]) or slightly branched and dispersible in water, forming gels in concentrations higher than 1.5% (Miles, Morris, Orford, & Ring, 1985). The AP is highly branched because of its additional bonds (α-D-[1 → 6]) and does not form gels (Banks & Greenwood, 1975). During gelatinization, starch granules irreversibly swell, AM selective leaching occurs, starch losses birefringence and crystallinity disappears. As a result, the swollen granules (deformable particles) get imbedded in a continuous matrix of cross-linked AM molecules (Lii, Shao, & Tseng, 1995; Tester & Morrison, 1990). This polymeric complex presents a viscoelastic behavior, and it forms a gel or paste during the dispersion cooling when non-waxy starch concentration is ⩾6% (Ring, 1985).

The formation of a gel or paste, which is a determinant of food texture, depends not only on the starch concentration but also on the structure of the swollen starch granule, the amount of AM and AP leached from the granule, the interactions between AM, AP and the granule, and the heating conditions such as temperature, time, heating velocity and shear stress (Morris, 1990; Ong & Blanshard, 1995; Yuan, Thompson, & Boyer, 1993).

For many years the amylose content has been used as a predictor of cooked rice quality. However, texture differences among cooked grains from rice cultivars of similar amylose content have been reported (Juliano, 1998). Cooked grains from high-amylose rice types are usually firm, dry and not adhesive, while those of low amylose content are more adhesive, moist and soft (Mossman, Fellers, & Suzuki, 1983; Sowbhagya, Ramesh, & Bhattacharya, 1987).

Stickiness has also been related to rice grain length, those of short grain being usually adhesive and the long grain ones being not adhesive (Mossman et al., 1983). Stickiness, defined as the cohesion strength at a surface

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level is thought to be an important parameter of cooked rice quality.

Taking into account that cohesiveness is attributed to AP and that rice grain structure is determined by the starchy component, both the gelatinization properties and the material leached out of the grain during cooking are expected to strongly influence its stickiness.

The aim of this work was to study the viscoelastic properties of starch pastes and gels, and the stickiness of cooked grains from seven novel argentine rice genotypes as well as to analyze the effect of the amylose content upon such properties.

2. Materials and methods

2.1. Samples

Milled grains from seven novel argentine rice genotypes were analyzed. They consisted of three cultivars of a high total amylose (TAM) content (EL PASO 144, SAN MIGUEL and H-144-7), two cultivars of an intermediate TAM content (PALMAR and RICO), and two waxy cultivars of a very low TAM content (W4109 and W4111), which are shown in Table 1. The samples were obtained from Estación Experimental INTA, Concepción del Uruguay, Entre Ríos, República Argentina. El Paso 144, H-144-7, San Miguel and Palmar were long grain rice cultivars, the remaining being short grain ones. Grains were processed and milled into flour at the Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, UNL, Santa Fe.

2.2. Starch preparation

Rice starches were prepared by briefly soaking milled grains at pH 2.2 (to inactivate amylases), followed by treatment with protease (Sigma, type XIV, 250 mg/100 g of flour) and 0.01% thiomersal at pH 7.6 for 24 h at 37 °C. Several washes followed by centrifugation at 800*g* for 10 min at room temperature were done.

Water-saturated butanol was used to extract lipids from granular starch. To this end, duplicate samples containing 1.0 g of native starch were weighted in screw cap tubes, to which solvent was added in a quantity enough to allow a 5 mm free space on top. Tubes were heated on boiling water for 2 h with manual shaking every 15 min. After cooling, tubes were centrifuged (800*g* for 10 min at room temperature), the solvent was decanted, and the extraction procedure was repeated at least twice with fresh solvent. Extracted starches were washed twice with water-free methanol, followed by water-free ether, and were air-dried overnight (Morrison & Laignelet, 1983).

Table 1
TAM content of rice starches from seven argentine genotypes[A]

Variety	TAM^n g/100 g of starch
EL PASO 144	28.6^a
PALMAR	20.7^b
RICO	19.6^b
H-144-7	26.8^a
SAN MIGUEL	28.1^a
W4109	2.1^c
W4111	1.3^c

^{n}, the mean value of triplicates. Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

[A] TAM values were taken from our previous work (Iturriaga, López, & Añon, 2004).

2.3. Soluble and insoluble amylose content determination in hot water

In order to determine the content of soluble amylose (SAM) of the native starches, extraction in hot water was performed as described by Shanty, Sowbhagya, and Bhattacharya (1980), followed by iodine staining and spectrophotometric measurement as described by Juliano et al. (1981a). Determinations were carried out in triplicate. Potato amylose (Sigma Type III) and defatted W4111 were used as standards (the last one to prevent amylopectin interference). Absorbance was measured at 620 nm in a Unicam UV/Vis 310 spectrophotometer.

Insoluble amylose (IAM) content in hot water was calculated by subtracting the SAM content from the TAM value (IAM = TAM − SAM).

2.4. Rice grain cooking method

Rice cooking was performed as follows: 10 g of crude rice and 100 ml of water were subjected to constant boiling for 15 min. Cooking water was eliminated and cooked rice was allowed to cool for 2 h.

2.5. Gels preparation

In order to obtain reproducible pastes, native starch and distilled water were used. Duplicate samples of different concentrations (5%, 10%, 15% and 20% w/w) were prepared and gelatinization was performed over boiling water (95 °C) with gentle stirring. Cooking time was adjusted to ensure that all granules were gelatinized (i.e., birefringence was lost). To test for birefringence, the presence of the typical Maltese cross was assessed in a Leitz Ortholux II optical microscope, illuminated by polarized light and using a Leitz Vario Orthomat photographic camera.

After testing several cooking times, a 3 min cooking was selected since it resulted in the loss of birefringence while maintaining the integrity of almost all granules. Granules were cooled in water bath at 20 °C for at least 2 h before use.

2.6. Rheology

Dynamic assays were carried out in a Haake CV20 rheometer, with a parallel plates system and a gap of $h = 1$ mm, at 20 °C. Sweepings of deformation between 0% and 40% were performed to detect the linear zone at a frequency (ω) of 1 Hz. The Haake osc 2.0 software was used to collect data for parameters determination.

To characterize the viscoelasticity degree of samples at different time scales, frequency sweepings were performed maintaining a fixed deformation $\gamma = 10\%$. Duplicate values of the modulus G^*, G', G', η^*, and tg θ were determined in both sweepings. In order to assay the differential viscoelastic behavior of starch pastes, $G'_{\max}$, which is the elastic modulus corresponding to $\gamma_{\max}$ (limit value of linear viscoelasticity), was determined through deformation sweepings.

2.7. Stickiness

Stickiness was measured by the method proposed by Mossman et al. (1983), in which part of the Instron universal texture profile is used. Octuplicates of 2 g of cooked rice were prepared in plastic, cylindrical and bottomless test tubes of 2 cm diameter, which were placed at the center of the lower platform to prevent grains from running over the headstock when reaching the final position. Such a position was adjusted to 3 mm from the platform to avoid grain breaking by compression.

A 50 N headstock and a velocity of 10 mm/min were used. Once the headstock downwards running finished, it was maintained in that position for 10 s before starting the upwards running. Data were collected through a register. The curves obtained were turned into parameters (x, y) through the Ungraph processor 2.0, which transformed data into ASCII language. Data were gathered and integrated through Microcal Origin 6.0 software.

Stickiness values were expressed in Newton cm, applying the corresponding scale factors, Newton being the adhesiveness strength and cm the distance traveled by the headstock.

2.8. Statistical analysis

All determinations were carried out in replicates as indicated. Data were evaluated by using the one-way analysis of variance (ANOVA). Tukey's test of multiple comparisons was employed to compare mean values when the significant variance was found by ANOVA. Significance was established at a level of $p < 0.05$. The Statgraphics statistical analysis package was used.

3. Results and discussion

3.1. Soluble and insoluble amylose in hot water

Results corresponding to the amylose soluble fraction determination (SAM) and the calculated IAM values are shown in Table 2. Experimentally, the TAM measurement is carried out in macromolecular solution, the defatted starch being treated with hot 2 N NaOH, while the SAM is determined from a hot water extract of starch, in which swollen granules and fragments of gelatinized granules remain insoluble. In this system, the IAM would correspond to amylose and/or amylopectin long external chains, not leached out from the granule and capable of forming complexes with iodine when treated with chaotropic solvents such as NaOH (Chinnaswamy & Bhattacharya, 1986).

Table 2
SAM and IAM content of rice starches (g/100 g starch)

Variety	SAM^A g/100 g starch	IAM^A (TAM – SAM) g/100 g starch
EL PASO 144	13.7^a	14.9
PALMAR	9.6^b	11.1
RICO	12.9^a	6.6
H-144-7	13.1^a	13.7
SAN MIGUEL	13.2^a	14.9
W4109	0^c	2.1
W4111	0^c	1.3

^{A} is the mean value from four replications. Letters placed as superindices indicate the existence of homogeneous groups for species with the same letter to a level of significance $p < 0.05$, according to the Tukey's test of means differences.

El Paso 144, Rico, H-144-7 and San Miguel cultivars formed a homogeneous group of high SAM content. Palmar presented the lowest SAM value among the non-waxy genotypes. The waxy genotypes W4109 and W4111 did not register SAM content at all. Amylose solubility reached 46–48% of TAM in non-waxy genotypes (except for Rico, in which the value was 66%).

3.2. Viscoelastic behavior of undefatted rice starch pastes and gels through dynamic rheometry

3.2.1. Effect of concentration and starch amylose content upon the system elasticity

The increase in concentration brought about an increase in the elastic properties (evaluated through $G'_{\max}$), similar to that reported for starches of other origins (Radhika Reddy, Subramanian, Zakiuddun, & Bhattacharya, 1994; Tsai, Li, & Lii, 1997). Such an effect was less marked for waxy genotypes, which also showed elasticity degrees substantially lower than those of the non-waxy genotypes (Fig. 1).

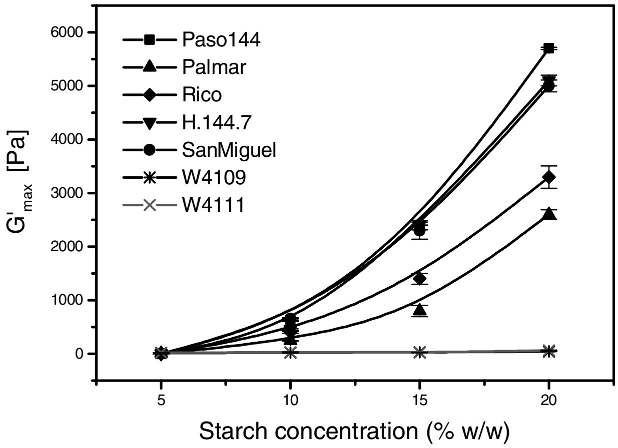

Fig. 1. Mean values of $G'_{\max}$ versus starch concentration (% w/w).

The variation of the elastic modulus with concentration followed the power law ($G'_{\max} \propto C^n$). The "n" values were 1.1 for waxy genotypes and 2.9–3.2 for the non-waxy ones, which were in line with the $n = 7$ reported for pure amylose gels within a range of concentrations of 1.5–7% w/w (Noel, Ring, & Whittman, 1993), and were similar to $n = 2.6$–3.2 for non-waxy rice starches (Biliaderis & Juliano, 1993; Lai, Shen, Yeh, Juliano, & Lii, 2001; Lii et al., 1995).

Non-waxy genotypes were unstable in 5% w/w suspensions (data not shown), because the minimum starch concentration required to obtain firm pastes is 6% (Lii et al., 1995; Ring, 1985). On the contrary, the waxy genotypes showed a stable behavior and a fluid aspect.

The decrease in the tg θ with increasing concentrations (Table 3) was also reported by Lii et al. (1995), and Radhika Reddy et al. (1994), for non-waxy rice starch, and by Hansen, Hoseney, and Faubion (1995) for corn starches.

The increase in the elastic modulus and the decrease in the tg θ with increasing concentration could be explained by the fact that during gelatinization the starch granules of the most concentrated systems would absorb all the water for swelling and form close packed systems, whereas in more diluted systems the remaining water would act as a dispersing phase, thus producing a paste with a predominantly viscous behavior (viscoelastic fluids) (Radhika Reddy et al., 1994).

In our gelatinized starch pastes, elasticity had a linear direct relation with the starch TAM content increase (Fig. 2), indicating that the gel-type structures formed by these systems upon cooling depend on the possibility to form an amylose network. As expected, waxy rice starch pastes (W4109 and W4111) of very low TAM content exhibited very low elasticity and a linear viscoelasticity zone extended within the range of the studied deformations (in this case, up to 100%). This behavior evidenced that the structure did not form in these systems, or it was very weak (rheogram not shown).

Table 3
Mean values of tg θ of starch gels from H-144-7, Palmar and Rico for different concentrations, taken at values of $\gamma = 10\%$ and $\omega = 1$ Hz

Genotype	Concentration (% w/w)			
	5	10	15	20
H-144-7	1.50[a]	0.70	0.20	0.10
PALMAR	0.70[a]	0.30	0.20	0.15
RICO	0.87[a]	0.44	0.17	0.12

[a] Unstable systems.

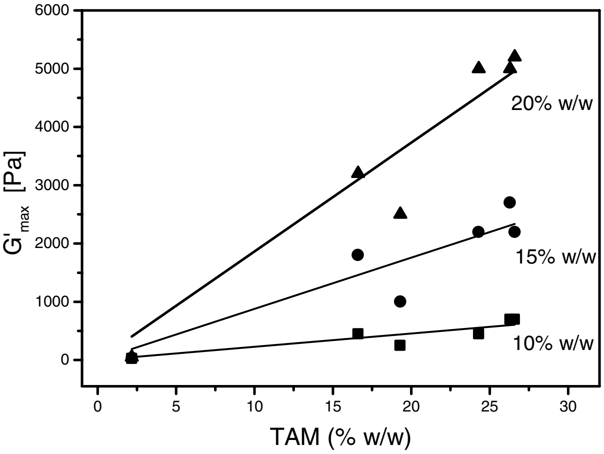

Fig. 2. Effect of TAM content on the $G'_{\max}$ value for 10%, 15% and 20% w/w starch concentrations.

However, as shown in Figs. 1 and 2, gels corresponding to Palmar rice were less elastic (and statistically different) than those of Rico, even though both presented the same amylose–amylopectin ratio. SAM measurements performed in an attempt to explain this difference confirmed that Palmar presents a lower SAM content than Rico. This may indicate that, within the range of concentrations studied in this work, the fraction leached out from the granule determines the development of the elastic properties.

Table 2 also shows that although Rico SAM content did not significantly differ from that of the high TAM genotypes (El Paso 144, H-144-7 and San Miguel), its gels were less elastic. This might be related to the IAM content, which was lower than that of the remaining non-waxy genotypes. Table 4 shows the correlation coefficients "r" between the $G'_{\max}$ value and the different amylose fractions content. A correlation was observed for the three fractions, and except for SAM, it was better for higher concentrations. Correlation was better for IAM than for SAM, which might reflect the importance of the insoluble fraction for conferring structure. Radhika Reddy et al. (1994) reported lower correlation coefficients for SAM, and similar ones for TAM and IAM.

These results allow us to postulate that the viscoelasticity of the pastes and starch gels from these seven genotypes depended on starch concentration and the TAM, SAM and IAM contents. The AM capacity to form union zones stabilized by hydrogen bonds confers rigidity to the granule when amylose is inside of it (IAM); when solubilized in the continuous phase (SAM), such capacity confers AM the ability to form a network whose strength and number of bonds depend on concentration. Our results also suggest that in genotypes having the same content of TAM, AM interactions in the continuous phase would prevail over AM interactions within the granules (IAM) in conferring elasticity to these gelatinized starch–water systems.

It follows that the final elasticity is the sum of the effect of granules rigidity and the network strength or resistance

Table 4
Correlation coefficients between $G'_{\max}$ values and TAM, IAM and SAM content for systems of different concentrations

	"r"[a] (×1000) value		
	Concentration (% w/w)		
	10	15	20
TAM	940	944	977
IAM	910	926	955
SAM	841	870	825

[a] The "r" values were significant at $p < 0.05$.

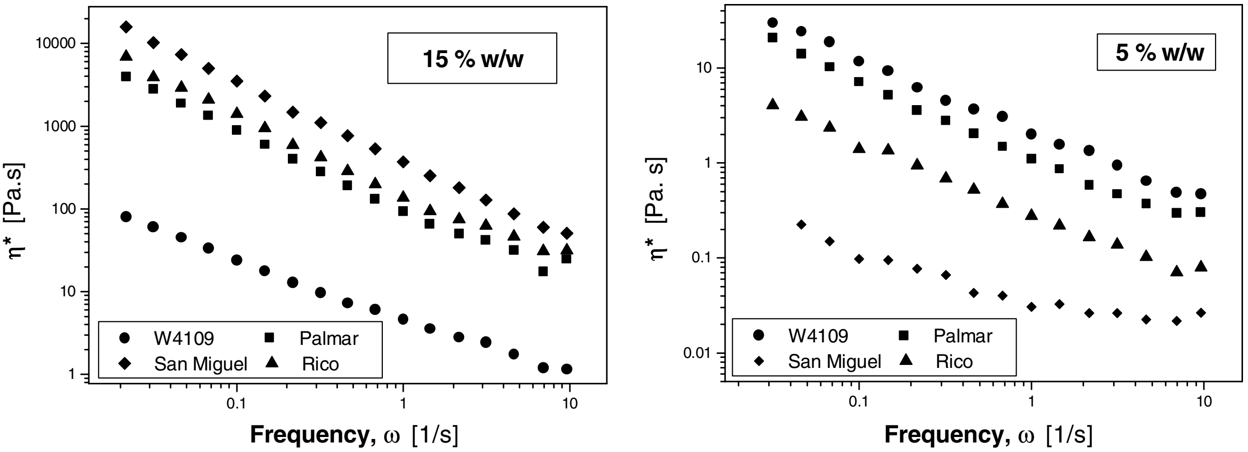

Fig. 3. Complex viscosity as a function of frequency corresponding to four genotypes for 15% w/w and 5% w/w concentrations.

due to the soluble components (Tsai et al., 1997). In addition, the interaction capacity of AM not only determines self-association, but also the formation of double helices between AM and external branches of amylopectin, and the interaction between these fractions and the integral granules. Interactions between granules would be also possible, which makes the system even more complex (Klucinec & Thompson, 1999; Ong & Blanshard, 1995).

3.2.2. Effect of amylose content on the complex viscosity of starch gels and pastes

The effect of AM and AP relative concentration during the continuous phase was evaluated through the complex viscosity, within the range of linear viscoelasticity values, which stands for the total resistance opposed by the fluid to the dynamic strength. Such a resistance is due to the interaction and/or friction between solute and solvent molecules and between solute molecules themselves. In this respect, resistance increased with starch concentration in all genotypes, and diminished with increasing frequencies (Fig. 3).

The complex viscosity also showed a direct relation to the TAM content, since a linear molecule has a higher hydrodynamic ratio and generates a higher excluded volume than a branched molecule of the same molecular weight.

Because AM and AP are thermodynamically incompatible, there is an inversion point (15: 85; w: w; AM: AP) below which viscous properties due to an AP-enriched continuous phase predominate. On the contrary, elastic properties predominate over the inversion point, as long as the AM concentration is sufficient ($C^* > 1.5\%$) (Ring, 1985). The effect of phase inversion is shown in Fig. 3.

In the highest concentration system (15%) η^* was proportional to the SAM content, whereas this pattern was inverted for 5% w/w concentrations. This effect was clearly observed with genotypes of intermediate TAM, Rico and Palmar. While Rico presented a behavior similar to that of high TAM genotypes, Palmar behaved as low TAM genotypes in 5% concentration systems. Since the properties of the continuous phase are mainly assessed in these diluted systems, the difference in SAM content would explain the differential behavior between both genotypes.

In the most concentrated systems, the continuous phase inversion effect (e.g., the predominance of AP cohesive and viscous properties) would be masked by the elasticity due to jellified AM and the granules rigidity.

These results are consistent with those reported by Sandhya Rani and Bhattacharya (1989) for rice flour pastes under different concentrations, temperatures and cooking times. They observed that paste viscosity was inversely proportional to the rice AM content at low concentrations (<7%), but was directly proportional at high concentrations (>7%).

4. Cooked rice grains stickiness[1]

Results were obtained by integrating the negative area of a typical INSTRON profile, and the stickiness value was expressed as Newton (N) per cm (or time in s). Data are shown in Table 5. The highest stickiness was observed for W4109, Rico and Palmar genotypes; H-144-7, while San Miguel showed intermediate stickiness and El Paso 144 was not sticky at all.

Stickiness has been associated with the type of rice (*japonica* or *indica*) and with the grain size, and it has been usually assumed that *japonica* rice is more adhesive than the *indica* one (Mossman et al., 1983). All the genotypes included in the current study were of the *indica* type; Rico and W4109 were short grain rice types, and the remaining genotypes were of long grain.

No relation was observed between grain size and stickiness, since long grain varieties such as H-144-7 and San Miguel presented intermediate values, while Palmar, which is also a long grain variety, was very sticky compared to El Paso 144.

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[1] This attribute was measured in six out of seven genotypes, because grains from the W4111 genotype were not available.

Table 5
Stickiness in six cooked rice varieties in excess of water

Genotypes	Stickiness^A X = [N cm]	σ	% VC
El PASO 144	0.18^a	0.04	22.2
PALMAR	0.74^b	0.10	13.5
RICO	0.82^b	0.10	12.2
H-144-7	0.42^c	0.06	14.3
SAN MIGUEL	0.42^c	0.07	16.6
W4109	0.86^b	0.11	12.8

^{A} Data stand for the mean values from eight replications. Mean values were compared by Tukey's test. The significant differences $p < 0.05$ between means from the same column are expressed through different superindices. The % VC is consistent with those reported by the literature (Mossman et al., 1983).

4.1. *Effect of amylose content on cooked rice grains stickiness*

Fig. 4 shows the relation between the stickiness of cooked milled rice grains and TAM and SAM content. An important decrease in stickiness is observed once a minimum AM content is surpassed.

If one assumes that a cooked grain is a gelatinized starch mass (since this component constitutes 90% of the grain, dry base), the SAM turns out to be of a particular importance as far as stickiness is concerned. This is so because the AM fraction leached out from the starch granule solubilizes, jellifies and minimizes the cohesive effects attributed to the branched fraction (AP), insofar as it breaks the AP molecules interactions (Klucinec & Thompson, 1999).

Therefore, an inverse correlation between SAM and stickiness is expected to occur. This is the case for El Paso 144 and W4109 genotypes. However, despite having a high SAM content, Rico exhibited a stickiness level similar to that of Palmar, whose SAM is lower, and to W4109, whose SAM is nil. On the other hand, H-144-7 and San Miguel, with a SAM content similar to that of El Paso 144, presented significant differences in their stickiness.

A negative linear correlation between TAM content and stickiness was reported by Juliano et al. (1981b), and by Sowbhagya et al. (1987), which is in line with our results for some genotypes (Fig. 4). El Paso 144, with a higher TAM content, was not sticky, and W4109 was the stickiest. Nevertheless, the remaining genotypes showed an inverse, not lineal relation.

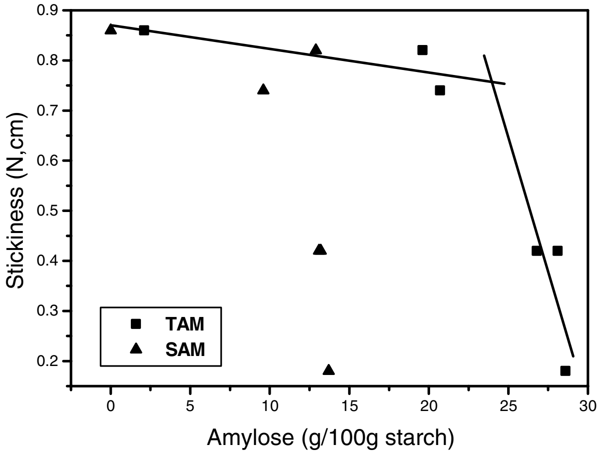

Fig. 4. Relation between TAM and SAM and the stickiness value of cooked rice grains from six genotypes.

It can be observed (Fig. 4) that stickiness substantially diminishes for TAM values higher than approximately 23.9% (for these six genotypes), whereas there is no variation for intermediate and low TAM contents.

Since TAM, SAM and IAM contents explained only partially the stickiness of these six genotypes, it is evident that other factors should be taken into account. Since TAM is the sum of SAM and IAM, the stickiness could be due to the soluble fraction, the insoluble fraction or both. Studies on the effect of proteins (glutelin) revealed that the binding of glutelin to starch components was directly related to the stickiness of cooked rice (Chrastil, 1990). Therefore, studies on the interaction of proteins with the soluble fraction of starch and their correlation with stickiness are warranted.

5. Conclusions

Starch pastes are complex systems in which viscoelastic properties deviate from the behavior expected for a two-components system (e.g., amylose:amylopectin), basically because of the presence of swollen grains. Nonetheless, it was possible to observe the effect of the amylose soluble fraction on elasticity in all studied concentrations. Extrapolation of these results to cooked grains explained only partially their stickiness, suggesting that additional assays are needed to evaluate properties and/or interactions on the surface of rice granules.

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