ANNEALING OF UNFERMENTED (*POLVILHO DOCE*) AND FERMENTED (*POLVILHO AZEDO*) CASSAVA STARCHES

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The effects of annealing on the properties of unfermented (*polvilho doce*-PD) and fermented (*polvilho azedo*-PAD) cassava starches were studied. The samples were submitted to annealing treatment for 24, 72, 96, 144, 192 and 240 hours. The annealing treatment changed the main physicochemical (gelatinization, enthalpy and temperature) and rheological properties (swelling power, peak viscosity and pasting temperature) of the both samples. The increase in the gelatinization temperatures and the narrowing of the gelatinization range was attributed to an reinforcement in the packing of the double helices of the amylopectin. The PAD samples seemed to be more affected by this treatment than PD ones. The PAD samples presented some characteristics of a waxy starch, such as increased crystallinity and increased stability of the pastes, which from a technological point of view is very important to diversify further its use in the food industry.

KEY-WORDS: CASSAVA STARCH; "POLVILHO DOCE"; "POLVILHO AZEDO"; ANNEALING.

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1 INTRODUCTION

The cassava (*Manihot esculenta Crantz*) belongs to the *Euphorbiaceae* family and is the main source of carbohydrates on the food of some developing countries. Depending on the manufacturing process of cassava starch, it can be named as "*polvilho doce*" (unfermented) or "*polvilho azedo*" (fermented). The first is obtained after the grating of the fresh roots, sedimentation and drying at sun. On the latter, the roots are grated and the pulp left to ferment naturally for 30 to 40 days (CEREDA, 1987).

The starches can have their physicochemical properties modified by several physical and chemical treatments. Physical treatments as annealing and heat-moisture (HMT), just use heat and water, but they modify significantly the properties of the starches (LIM, CHANG and CHUNG, 2001; VERMEYLEN, GODERIS and DELCOUR, 2006). The first observations of the effects of annealing on starch were probably reported by Gough and Pybus (1971). Annealing is generally carried out by heating granular starch with a large quantity of water, at temperature below the starch melting point, whereas HMT is carried out at limited moisture contents but at an elevated temperature (ELIASSON and GUDMUNDSSON, 1996). In this process the arrangement of the macromolecules undergoes subtle changes resulting from rearrangement of hydrogen bonds which are broken during the gelatinization process and reformed as the granule components attain a state of more ordered crystal conformation.

The annealing modifies the physicochemical properties of starch without destroying its granular structure (LARSSON and ELIASSON, 1991; JACOBS and DELCOUR, 1998). These modifications however, depend on temperatures, time of treatment, starch to water ratio, botanical species and manufacturing process of starch. The annealing process decrease the swelling power and solubility of starches (EERLINGER, JACOBS and BLOCK, 1997), increase the gelatinization temperatures and enthalpy of treated starches (STUTE, 1992; TESTER and DEBON, 2000), increase the susceptibility to amilases (WANG, POWEL & OATES, 1997) and change the curves of development of pasta (STUTE, 1992).

Jacobs et al. (1995) reported an increased gelatinization temperature and enthalpy, a narrower gelatinization temperature range, and an increase in peak viscosity and viscosity upon cooling for pea, wheat and rice annealed for 24 hours. Annealing of potato starch does not (KARLSSON and ELIASSON, 2003) or only moderately (NAKAZAWA and WANG, 2003; TESTER et al., 2005) increase enthalpy of gelatinization. Starch annealing has been sugested to induce crystal growth or perfection, although it does not increase crystallinity (TESTER *et al.*, 2005).

The aim of this study was to compare the effects of annealing treatment on the properties of *polvilho azedo* and *polvilho doce*.

2 EXPERIMENTAL

2.1 ANNEALING PROCEDURE

The annealing treatment of the commercial samples of *polvilho doce* (PD) and *polvilho azedo* (PAD) were obtained after steeping in water (1:5 starch/water) to $50^{\circ}C$ for 24, 72, 96, 144, 192 and 240 hours, according to Gough and Pybus (1971).

2.2 SWELLING POWER AND SOLUBILITY

The swelling power and solubility analysis were determined by Schoch methodology (SCHOCH, 1964). About 1 g of sample and 40 mL of water were submitted to heating to temperatures of 55, 65, 75, 85 and $95^{\circ}C$. After cooling, the slurry of starch was centrifuged 5000 x g for 30 min. The supernatant was carefully separated from the sediment, and 10 mL were removed to evaluate the percentage of soluble, while the sediment was weighed for determination of the percentage of swollen granules.

2.3 RAPID VISCO-ANALYZER (RVA)

The pasting characteristics of the starches were analyzed in a rapid visco-analyzer (RVA) model RVA-4 (Newport Scientific Pty. Ltd, Australia) was used for pasting properties determinations. About 4,5 g of sample were mixed with 25 mL of distilled water to obtain a total weight of 28 g in the glass of RVA. The analyses were run in triplicate. The samples were maintained for 1 min at 50°C and the temperature increased progressively up to 95°C (13°C/min), held for 3 min at 95°C and afterwards reduced to 50°C (13°C/min), held for 3 min. The test was concluded after 13 min (NEWPORT SCIENTIFIC, 1998).

## 2.4 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Thermal characteristics of the starches were obtained by analysis in a differential scanning calorimeter Shimadzu, model DSC-50 (Shimadzu, Japan). Approximately 3 mg of sample were placed into an aluminium sample pan, 15 µL of distilled water was added to obtain a starch to water ratio of 1:3 (w/v). The samples were allowed to equilibrate for 1h and pans were hermetically sealed. The calorimeter was heated at 25-100°C, under gas flow of 50 mL/min and heating rate was to 10°C/min. The parameters of T_o (temperature of onset gelatinization), T_p (peak temperature), T_c (conclusion temperature) and ΔH (enthalpy of gelatinization) were obtained (SHIMADZU CORPORATION, 1989).

2.5 X-RAYS DIFFRACTION

The X-rays patterns of the starches were obtained in an x-ray diffractometer Rigaku, model Dmax-B (Rigaku, Japan), with a copper radiation in line $K\alpha$ (λ = 0.1542 nm), operating at 40 KV and 25 mA. The area of scanning of diffraction angle (2θ) was 3-40° (1/2°/min). The diffractograms were fitted in Origin 6.0 software (MICROCAL INC., 1996).

2.5.1 Relative Crystallinity

The relative crystallinity was determinate according to Herman's methods, as described in Fujita et al. (1998). A smooth curve which connected peak baselines was computer-plotted on the diffractograms obtained in x-ray analysis (Figure 1). The area above the smooth curve was taken as the crystalline portion and the lower area between smooth curve and the linear baseline which connected the two points of the intensity 2θ of 30º and 5º in the samples was taken as the amorphous section. The upper diffraction peak area and the total diffraction area over the diffraction angle 5°-30° 2θ were integrated using Origin 6.0 software (MICROCAL INC., 1996). The ratio of upper area to total diffraction was taken as the degree of crystallinity.

Diffraction angle (2θ)
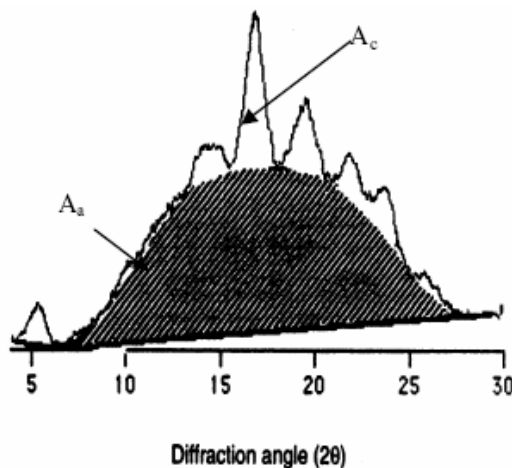
FIGURE 1 - DIAGRAM FOR CALCULATION OF THE DEGREE OF RELATIVE CRYSTALLINITY

The equation of the degree of crystallinity is as follows: Xc = Ac/(Ac + Aa)
Where Xc refers to the degree of crystallinity; Ac refers to the crystallized area on the X-ray diffractogram; Aa refers to the amorphous area on the X-ray diffractogram.

3 RESULTS AND DISCUSSION

3.1 PASTING CHARACTERISTICS

The effects of the annealing on the pasting properties of the *polvilho doce* (PD) and *polvilho azedo* (PAD) are shown in the Table 1 and Figures 2 and 3. There was reduction in the peak viscosities of the two samples by annealing time. The samples of PD and PAD annealed by 144-240 h suffered the reductions more intensively. As already observed by other authors (HOOVER & VASANTHAN, 1994; GOMES et al. 2004), the treatment reinforces the bonds between the starch molecules, making the amylose less prone to leach from the granules, which causes a decrease in the peak viscosity of the treated samples.
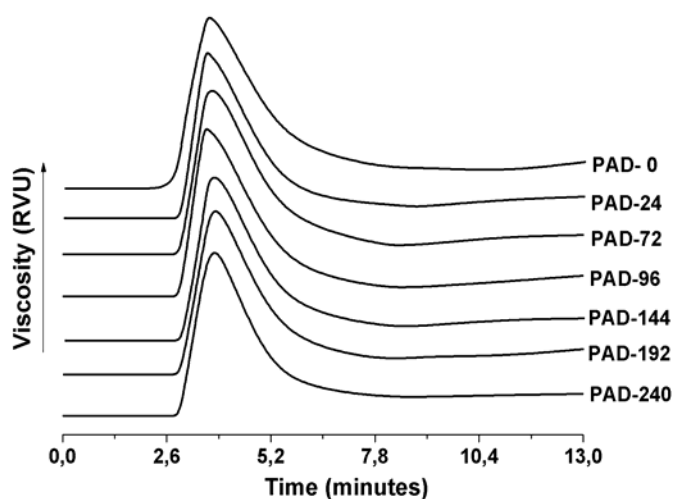

FIGURE 2 - RVA CURVES OF NATIVE AND ANNEALED PAD STARCHES
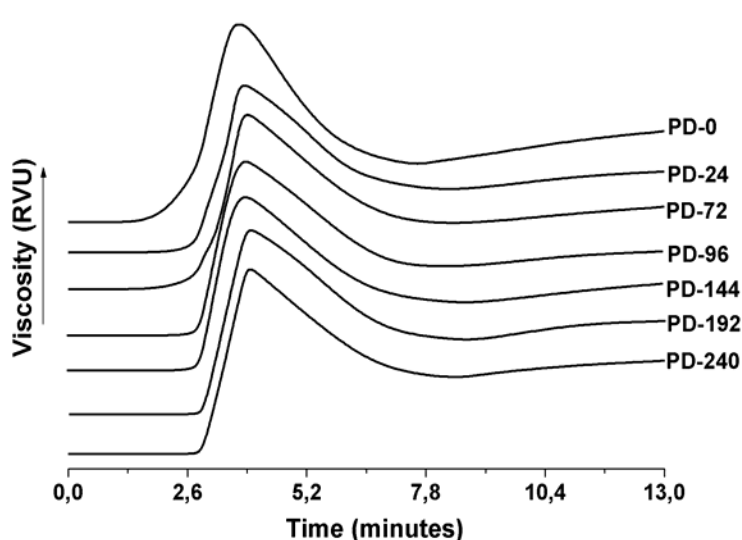

FIGURE 3 - RVA CURVES OF NATIVE AND ANNEALED PD STARCHES

The fermentation process changed the associative forces between the molecules of PAD, which led it to a larger reorganization of its molecules in relation to PD. Thus the molecules seemed to assume a more stable conformation due to an increase in the crystalline order, which greatly influenced the pasting properties of the samples annealed. The untreated PAD presented a smaller setback than the PD. The latter however, suffered a more effective reduction in the setback (47,0 %) when the time of annealing was increased.

The cassava starch (fermented or unfermented) showed lower tendency to the retrogradation than the others starches as potato, corn, etc. The factors which probably contributed for this were the higher degree of polymerization (DP) and the lower lipids content. These can provoke a repulsive effect among the molecules, which prevented the association among them and decreased the formation of the amylose/lipids complexes.

TABLE 1 - EFFECTS OF ANNEALING ON THE PASTING CHARACTERISTICS OF THE SAMPLES OF PD AND PAD[1]

TIME	PD			PAD		
(h)	PV (RVU[2])	SB (RVU[2])	FV (RVU[2])	PV (RVU[2])	SB (RVU[2])	FV (RVU[2])
0	655,9 ±1,88[ab]	115,3±13,1[a]	299,7± 15,62[a]	251,2± 0,17[a]	13,6±0,25[a]	38,2±1,88[ ]
24	643,9 ±1,42[b]	69,0±4,36[bc]	302,6±7,02[abc]	266,8±0,80[b]	14,3±0,22[b]	35,5±1,26[b]
72	670,5 ± 1,80[c]	62,5±1,54[cd]	310,1±1,89[ab]	241,8±4,00[c]	14,6±0,13[b]	31,4±2,41[b]
96	658,4±2,79[ab]	56,7±3,22[d]	313,3±2,79[ab]	236,0±1,98[c]	15,4±0,63[b]	31,1±2,98[b]
144	594,2±6,69[d]	63,5±3,55[cd]	297,6±2,07[ac]	176,9±1,10[e]	11,4±0,05[c]	22,1±0,46[ ]
192	613,1±3,45[e]	60,9± 0,60[d]	307,9±0,60[ab]	183,1±2,00[f]	14,5±0,79[ab]	27,8±,87[c]
240	631,9±1,48[f]	61,2±3,25[cd]	314,5±4,58[ab]	177,7±0,70[e]	12,4±0,30[d]	23,4±0,54[ ]

[1]Equal letters in the same column do not differ significantly at the 5%.
[2]RVU = Rapid Visco-analyser Unity.
PV = peak viscosity, SB = setback, FV = final viscosity.

3.2 SWELLING POWER AND SOLUBILITY

The annealing restricted the swelling and the solubility of the two samples in all temperatures by the time of treatment. This can be verified in Table 2. At 55°C, the solubility of the samples of PD ceased after 144h of treatment, while in the PAD annealed for 240h, the solubility was reduced by 94,1%. The solubility at 95°C of the PD and PAD were reduced to 30,6% and 12,3%, respectively, in relation to the control sample.

The swelling of the samples annealed by 240h at 55°C were restricted in 15,4% (PAD) and 47,8% (PD), in relation to control samples. While at 95°C the reductions were of 43,1% and 38,5%, respectively. The two fractions of amylose present in the granule, lipid-amylose complex (LAM) and free lipids amylose (FAM), interfere directly in the swelling and solubility of the starches.

FAM, which prevails in the cereals and tubers starches, interferes in the formation of the amylopectin crystals and consequently facilitates the swelling of the amorphous areas, causing their destabilization. The annealing treatment corrects this destabilization, by inducing interactions of FAM with the amylopectin molecule (forming a more stable structure), increasing the perfection of the crystals, hindering the hydration of the amorphous areas, reducing thus the solubility and the swelling as annealing time is increased.

# TABLE 2 - EFFECTS OF ANNEALING ON THE SWELLING POWER AND SOLUBILITY OF PD AND PAD AT 55ºC AND 95ºC[1]

	PD				PAD			
TIME	SWELLING POWER (%)		SOLUBILITY (%)		SWELLING POWER (%)		SOLUBILITY (%)	
(h)	55 ºC	95 ºC	55 ºC	95 ºC	55 ºC	95 ºC	55 ºC	95 ºC
0	4,6± 0,04[a]	41,8 ± 0,31[a]	0,89± 0,00[a]	16,0± 0,11[a]	2,6± 0,02[a]	21,3± 0,27[a]	1,9± 0,64[a]	26,8± 0,26[a]
24	2,8± 0,01[b]	29,0± 0,01[b]	0,56± 0,02[b]	12,0± 0,17[b]	2,5± 0,06[bc]	20,3± 0,21[b]	1,2± 0,01[b]	25,4± 0,24[b]
72	2,7± 0,01[c]	27,5± 0,54[c]	0,22± 0,00[c]	11,7± 0,41[b]	2,4± 0,02[bd]	18,2± 0,22[c]	1,1± 0,02[c]	24,9± 0,29[cd]
96	2,7 ± 0,02[c]	27,6± 0,12[c]	0,11± 0,00[d]	11,7± 0,01[bc]	2,4± 0,01[bd]	17,3± 0,13[d]	1,1± 0,01[c]	24,2± 0,23[d]
144	2,5± 0,01[d]	25,8± 0,27[d]	*	11,4± 0,01[bc]	2,3± 0,01[e]	15,4± 0,32[e]	0,7± 0,01[d]	24,6± 0,34[cd]
192	2,5± 0,00[d]	25,8± 0,77[d]	*	11,5± 0,01[bc]	2,2± 0,02[f]	15,7± 0,12[e]	0,4± 0,06[e]	23,7± 0,28[de]
240	2,4± 0,02[e]	23,8± 0,20[e]	*	11,1± 0,05[d]	2,2±0,03[f]	13,1± 0,30[f]	0,1± 0,01[f]	23,5± 0,13[e]

[1]Equal letters in the same column do not differ significantly at the 5%.
*Not detected.

3.3 GELATINIZATION TEMPERATURE

The results of the gelatinization analysis of the PD and PAD samples are shown in the Table 3 and Figures 4 and 5. All the annealed samples suffered increases in T_o, T_p and ΔH, in relation to the control sample. The gelatinization enthalpy (ΔH) of the PAD annealed for 240h increased 9,7% in relation to the PD annealed for the same time.
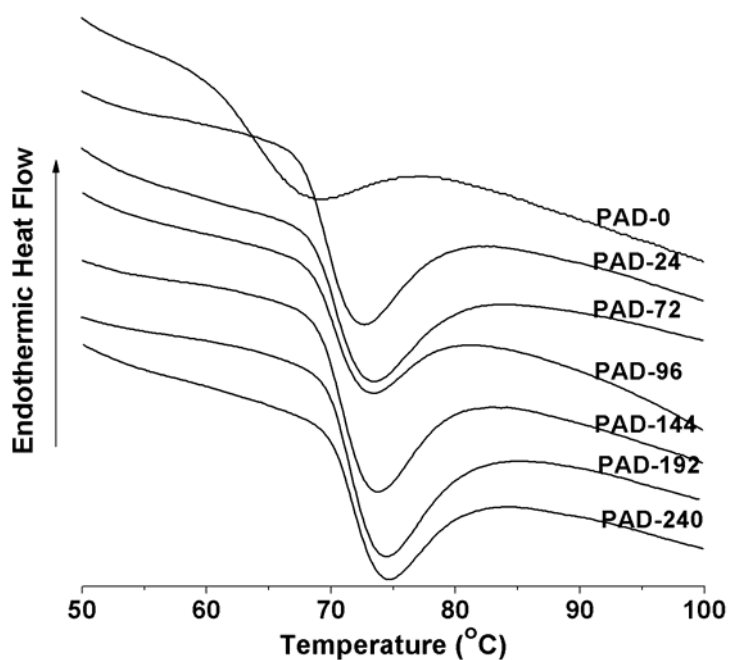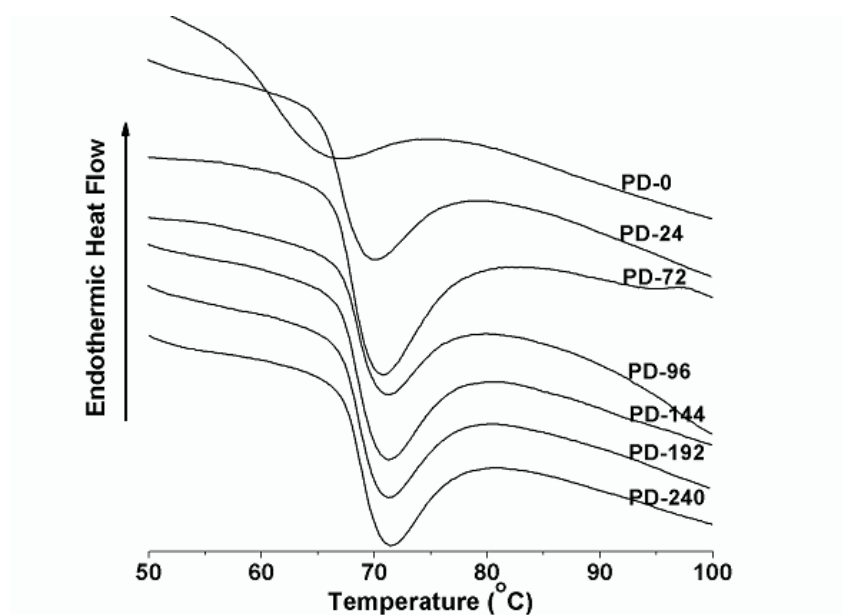

FIGURE 4 - DSC CURVE OF NATIVE AND ANNEALED PAD STARCHES

The increase in the gelatinization temperatures and the narrowing of the gelatinization range may have been caused by an alteration in the coupling forces among the amorphous and crystalline parts, as a consequence of the reinforcement in the packing of the double helices of the amylopectin.

TABLE 3 - EFFECTS OF ANNEALING ON THE GELATINIZATION CHARACTERISTICS OF THE SAMPLES OF PD AND PAD

TIME	PD				PAD			
(h)	T_O (^{O}C)	T_P (^{O}C)	ΔT	ΔH (J/g)	T_O (^{O}C)	T_P (^{O}C)	ΔT	ΔH (J/g)
0	58,6	67,1	13,3	5,1	61,7	69,4	10,2	3,3
24	65,7	70,2	10,0	6,1	67,1	72,2	10,3	9,1
72	66,6	70,9	11,3	8,2	68,4	73,2	11,4	12,4
96	67,1	71,3	10,2	5,4	68,7	73,3	10,1	7,2
144	67,2	71,3	9,7	7,1	69,1	74,0	10,1	7,5
192	67,4	71,4	10,0	7,6	70,0	74,7	11,2	11,1
240	67,3	71,5	10,0	7,6	70,0	74,7	10,1	8,4

Tp = peak temperature; ΔT = temperature range (Tc-To); ΔH = enthalpy.

**FIGURE 5 - DSC CURVE OF NATIVE AND ANNEALED PD STARCHES**

The enthalpy of gelatinization of PAD-0 was much smaller than the PD-0, which was probably a consequence of the fermentation process occurred in PAD-0, which become the links weaker and that may be broken under heating. For this reason the energy used to break them was lower.

The annealing treatment induced to a reorganization of the molecules, which provoked the increase in the enthalpy and temperatures of gelatinization. With the increase of time of treatment, the molecules of PAD seemed to have suffered a more pronounced rearrangement than the ones of PD, because the latter already has a higher degree of organization between its molecules.

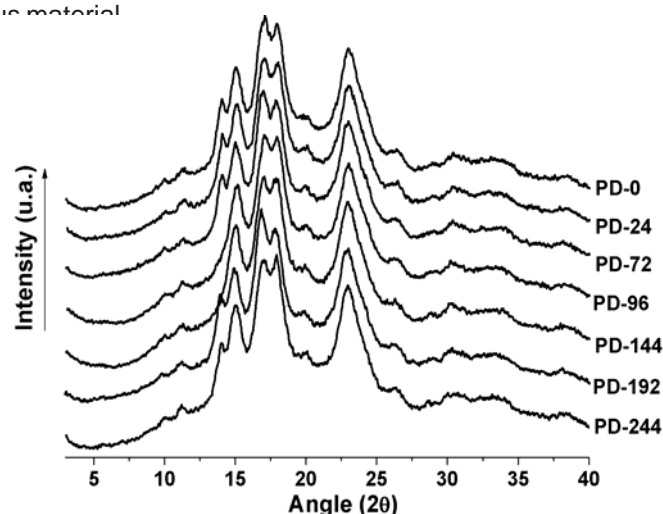## 3.4 X-RAY DIFFRACTION AND RELATIVE CRYSTALLINITY

The intensities in the main peaks diffraction and the relative crystallinities (RC) of the PD and PAD samples are presented in the Table 4 and Figures 6 and 7. There were no modifications in x-ray patterns of annealed samples, but the main diffraction peaks (14°, 15°, 17°, 18° e 23°) showed increase on diffraction intensity, principally in PAD samples.
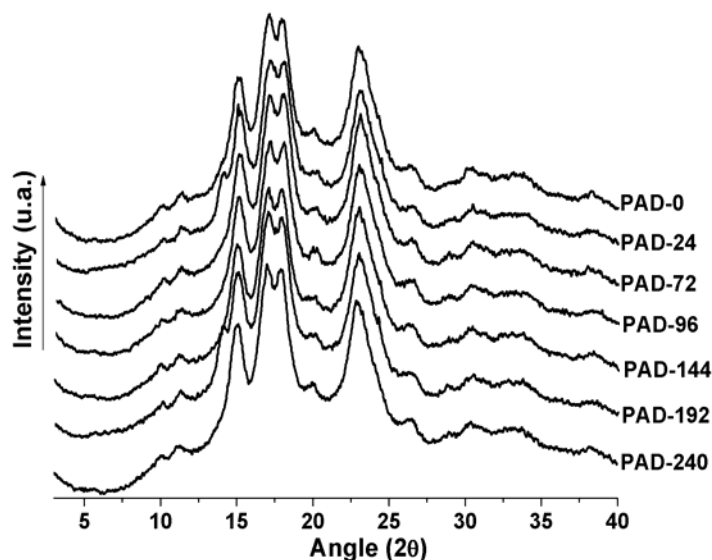
FIGURE 6 - X-RAY DIFFRACTION PATTERNS OF NATIVE AND ANNEALED PAD STARCHES

The PAD sample presented RC 3,0% larger than the PD. The PAD annealed by 240h presented intensity increased in the rings 17°, 18° and 23°, in relation to the control sample. The PAD sample annealed for 72h presented higher intensity in almost all the rings. The increase in RC after annealing indicates that the crystalline structures acquired a more compact and organized packing.

The unfermented and fermented cassava starches (PD and PAD) untreated presented an x-ray pattern type C_A and A, respectively. The C_A pattern is an intermediate between A and B pattern, but shows majors characteristics of type A. The C_A pattern shows main peaks at 14°, 15°, 17°, 18°, 20° and 23° (2θ). The A pattern shows main peaks at 15°, 17°, 18°, 20° and 23° (2θ). The PAD samples presented more narrow peaks, indicating that the hydrolysis during the fermentation process attacked the amorphous areas, leaving intact the crystalline ones. The PD samples presented broader peaks, indicating larger presence of amorphous material.

FIGURE 7 - X-RAY DIFFRACTION PATTERNS OF NATIVE AND ANNEALED PD STARCHES

TABLE 4 - EFFECTS OF ANNEALING ON THE INTENSITIES OF THE MAIN PEAK DIFFRACTION AND THE RELATIVE CRYSTALLINITIES OF THE PD AND PAD

	PD				PAD			
TIME	INTENSITIES (AU)[1]			RC	INTENSITIES (AU)[1]			RC
(h)	17°	18°	23°	(%)	17°	18°	23°	(%)
0	3441	3331	2988	17,4 ± 0,24	3417	3359	3018	17,9 ± 0,10
24	3233	3130	2854	17,1 ± 0,08	3398	3424	3026	19,2 ±0,01
72	3334	3199	2894	16,5 ±0,15	3429	3449	3241	19,3 ±0,07
96	3339	3272	2897	16,5 ±0,07	3384	3362	3040	18,5 ± 0,10
144	3328	3118	2773	16,6 ±0,08	3348	3320	3061	18,5 ± 0,17
192	3231	3250	2854	16,3 ±0,11	3514	3418	2995	18,7 ±0,05
240	3253	3319	2917	16,9 ±0,05	3448	3402	3032	18,7 ±0,02

[1]AU = arbitray unity.

4 CONCLUSION

The annealing treatment changed the physicochemical characteristics of the cassava starches. In almost all determinations, the PAD samples seemed to be more affected by this treatment than PD ones, because the latter already has a higher organization while in PAD, the bonds are weakened by fermentation process (hydrolysis of starch bonds) and hence they are more prone to reorganization. The PAD samples presented some characteristics of a waxy starch, such as increased crystallinity and increased stability of the pastes, which from a technological point of view is very important to diversify further its use in the food industry.

RESUMO

ANNEALING DOS AMIDOS DE MANDIOCA NÃO FERMENTADO (*POLVILHO DOCE*) E FERMENTADO (*POLVILHO AZEDO*)

Os efeitos do *annealing* nas propriedades dos amidos de mandioca fermentado (polvilho azedo - PAD) e não fermentado (polvilho doce - PD) foram analisados. As amostras foram submetidas ao tratamento *annealing* por 24, 72, 96, 144, 192 e 240 horas. O tratamento modificou as propriedades físico-químicas (entalpia e temperatura de gelatinização) e reológicas (poder de inchamento, temperatura de pasta e viscosidade máxima) de ambos os amidos. O aumento nas temperaturas de gelatinização e o estreitamento na faixa de gelatinização foram atribuídos ao fortalecimento no arranjo das duplas hélices da amilopectina. O PAD mostrou-se mais afetado pelo tratamento *annealing* que o PD. O polvilho azedo apresentou algumas características de amido ceroso, como aumento da cristalinidade e da estabilidade das pastas, o que sob o ponto de vista tecnológico é muito importante para diversificar ainda mais a sua utilização na indústria de alimentos.

PALAVRAS-CHAVE: AMIDO DE MANDIOCA; POLVILHO DOCE; POLVILHO AZEDO; ANNEALING.

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