Starch Identification and Determination in Sweetened Fruit Preparations. 2. Optimization of Dialysis and Gelatinization Steps, Infrared Identification of Starch Chemical Modifications

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Identification and quantification of starch used as thickening and gelling agent (TGA) in sweetened fruit preparations (SFP) are difficult because of the low levels of concentration and the heterogeneous, gelified, and highly sweetened nature of the medium. A method is proposed that overcomes those difficulties. In a first step, starches are identified by optical microscopy and acetyl and hydroxypropyl modifications are characterized by Fourier transform infrared spectroscopy (FT-IR). Starch quantification requires high purification, achieved by dialysis to remove most of the sucrose. The experimental conditions of this step are optimized. A previous gelatinization treatment, the conditions of which are studied here, improves significantly the enzymatic hydrolysis of starch and the quantification of the released glucose. The method proposed, applied to 15 different SFP, allows an accurate and repeatable determination of distarch phosphate, acetylated distarch phosphate, and acetylated distarch adipate. It provides, furthermore, an appropriate tool for the special case of a hydroxypropylated distarch phosphate.

Keywords: *Sweetened fruit preparation; Fourier transform infrared; dialysis; starch identification and determination*

INTRODUCTION

Sweetened fruit preparations (SFP) are industrial intermediate food products. Their main applications are in dairy products, but they are also used in confectionery, bakery, and pastry products and in ice cream, sorbet, and dessert manufacture. SFP are complex food matrices because of their heterogeneity (fruit pieces) and their gelified texture. The latter is caused by natural fruit pectin and the addition of exogenous thickening and gelling agents (TGA) such as modified starches. The analysis of these food additives is of interest regarding regulatory aspects. It is also a technological necessity because these TGA may modify the rheology of products to which SFP are incorporated.

The direct quantification of starch in SFP cannot be achieved from raw samples because of their high sucrose concentration (550 g/L) and the lack of specificity of commercial amylolytic enzymes preparations (Chatel et al., 1996). Indeed, a significant quantity of glucose is released from sucrose during starch hydrolysis. This phenomenon hinders the determination of the glucose issued from starch, which concentration is lower (6−35 g/L).

A recent study (Chatel et al., 1996) showed that a method based on purification by dialysis, gelatinization, hydrolysis, and enzymatic assay allowed the quantification of acetylated distarch adipate with accuracy and repeatability, in that type of sample. The application of this method extended to different modified starches in 15 SFP led to a thorough study of experimental conditions of dialysis and gelatinization. In that purpose, Fourier transform infrared spectroscopy (FT-IR) was a powerful tool to identify chemical modifications.

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Table 1. Thickening and Gelling Agents (TGA) Contents (Percent w/w) of the SFP

SFP	DP[a]	ADP[b]	ADA[c]	HDP[d]	pectin	guar	xanthan
SFP 1	0.5				0.3		
SFP 2	3.0				0.3		
SFP 3		0.5			0.3		
SFP 4		3.0			0.3		
SFP 5			0.5		0.3		
SFP 6			1.0		0.3		
SFP 7			2.0		0.3		
SFP 8			3.0		0.3		
SFP 9				0.5	0.3		
SFP 10				1.0	0.3		
SFP 11				2.0	0.3		
SFP 12				3.0	0.3		
SFP 13			2.9			0.6	0.1
SFP 14			2.9		0.7	0.3	
SFP 15			2.9		0.3	0.3	

[a] Distarch phosphate. [b] Acetylated distarch phosphate. [c] Acetylated distarch adipate. [d] Hydroxypropylated distarch phosphate.

MATERIALS AND METHODS

Samples. Four modified starches have been used: acetylated distarch adipate (ADA) Colflo 067, National Starch (Villefranche sur Saone, France); distarch phosphate (DP) Cleargel A, National Starch; acetylated distarch phosphate (ADP) C Tex 06306, Cerestar (Haubourdin, France); hydroxypropylated distarch phosphate (HDP) VA 70, Cerestar. Pectins (Genu 101 and 104 AS) were supplied by Copenhagen Pectin (Lille Skenved, Denmark). Sucrose (ref 84105) was a Fluka product (Buchs, Switzerland).

SFP were supplied by International Research Center Daniel Carasso, DANONE group (Le Plessis Robinson, France). SFP contained 50.0% strawberry (SFP1-SFP14) or apricot (SFP15), 45.0% sucrose, 0.1% (w/w) potassium sorbate, and the thickening and gelling agents described in Table 1. Samples were adjusted to 100% (w/w) with water. SFP were not pasteurized and potassium sorbate was used as preservative.

Moisture Content Measurement. Moisture content of starch samples was determined according to the French standard method NF V-03 707 (Afnor, 1976).

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Table 2. Experimental Field

	factor	lower limit (−)	upper limit (+)
U_1	membrane cutoff (Da)	2000	8000
U_2	water renewal (mL/min)	200	600
U_3	dialysis time (h)	8	24
U_4	temperature (°C)	25	35
U_5	starch (% w/w)	0.5	3.0
U_6	pectin (% w/w)	0.3	1.0
U_7	sucrose (% w/w)	23.0	46.0

Dialysis Protocol. Cellulose acetate molecular porous dialysis membranes (ref spectra por 6, MWCO 2000 or 8000 Da, Spectrum, Houston, TX) containing 50 mL of sample were maintained for 4−72 h in a water bath (volume 8 L) with a continuous removal of water controlled by a Brooks debitmeter (ref R-7M25L, Hatfield, PA) calibrated from 60 to 600 mL. SFP were diluted in water (1/1 w/w) before dialysis. Agitation and temperature (from 25 to 45 °C) were controlled by a thermoregulator (ref 86663, Polystat, Illkirsch, France).

Experimental Design. A research strategy (Box et al., 1978; Fargin et al., 1985) relying on two fractional factorial designs was used to optimized dialysis experimental conditions. All factors that could modify the dialysis efficiency were identified and estimated from literature data (Bernetti et al., 1990; Southgate, 1991; Tsuge et al., 1990) and personal results. In Table 2, the experimental field lists the main factors (U_i factors) and shows the upper and lower limits of the intervals studied. Test solutions (S_1−S_8) contained 23% (w/w) (S_1, S_4, S_6, S_7) or 46% (w/w) (S_2, S_3, S_5, S_8) sucrose, 0.5% (w/w) (S_3−S_6) or 3.0% (w/w) (S_1, S_2, S_7, S_8) starch, and 0.3% (w/w) (S_2, S_4, S_5, S_7) or 1.0% (w/w) (S_1, S_3, S_6, S_8) pectin. The complement to 100% (w/w) was obtained with water. The experimental conditions were established from experimental limits (Table 2) and from the structures of two fractional factorial designs defined by a relation of definition made of four independent generators.

design 1, relation of definition:

$$I \equiv 1.2.4 \equiv 2.3.5 \equiv 1.3.6 \equiv 1.2.3.7 \quad (1)$$

design 2, relation of definition:

$$I \equiv -1.2.4 \equiv -2.3.5 \equiv -1.3.6 \equiv 1.2.3.7 \quad (2)$$

The number n of experiments defined for each fractional factorial design was calculated from the numbers K of factors ($K = 7$) and r of independent generators ($r = 4$) by applying the following relation:

$$n = 2^{(k-r)} = 2^{(7-4)} = 8 \text{ experiments per design} \quad (3)$$

The use of an experimental strategy to optimize dialysis conditions gave workable results with only 16 experiments. This is lower than the theoretical number of 128 experiments necessary to test all of the possible combinations among the 7 factors of Table 2.

Experiments were achieved in randomized order to avoid block effects. Results calculated from eq 4 were expressed in percentage of elimination of sucrose ($T\%$).

$$T\% = \frac{[\text{sucrose}]_{\text{initial}} - [\text{sucrose}]_{\text{final}}}{[\text{sucrose}]_{\text{initial}}} \times 100 \quad (4)$$

The influence of each factor (U_i) on dialysis efficiency was estimated by working out its main effect (b_i) from multilinear regression of experimental results and from the structures of the two fractional factorial designs. In the same way, antagonistic and synergetic effects between factors were determined from the first-order interaction effects (b_{ij}). Interactions between more than two factors were considered to be negligible.

Gelatinization. Modified starches solutions (5.0 g/L) were gelatinized owing to a heat treatment at 20, 50, 60, 70, or 80 °C during 30 min under strong agitation in alkaline [1 mL of NaOH (5 M) in 50 mL of sample] or neutral medium. The dialyzed sweetened fruit preparations were alkalinized [1 mL of NaOH (5 M) in 50 mL of sample] at 70 °C under strong agitation during 30 min. The alkalinized samples were neutralized (pH value 6−7) by HCl (5 M).

Fourier Transform Infrared Spectroscopy (FT-IR). SFP were dispersed in boiling water (25 g in 25 mL) and filtrated on a strainer (∅ 0.5 mm) to remove fruit pieces and vegetal fragments. Starch was extracted from the filtrate by precipitation in hydroalcoholic medium (ethanol >80 % v/v). Dispersion and precipitation steps were repeated at least twice to improve the purity of the FT-IR samples. Samples were then centrifuged during 10 min at 600*g*, and the centrifugal pellets were vacuum-dried at room temperature.

The modified starches FT-IR spectra were obtained after pelletizing (1% w/w) in KBr (ref 60090, Fluka), using a FT-IR spectrophotometer (ref 16PC-IRTF, Perkin-Elmer, Norwalk, CT) coupled with a personal computer loaded to a spectra processing sofware (IRDM, Perkin-Elmer). Nine scans were made for each spectrum with a 4 cm^{-1} resolution.

Hydrolysis and Enzymatic Assays. Hydrolysis and enzymatic assays of sucrose and starches were performed as previously described (Chatel et al., 1996).

RESULTS AND DISCUSSION

Study of Experimental Conditions of Dialysis. The interferences to starch quantification induced by sucrose can be reduced by dialysis.

This simple and efficient technique to purify thickening and gelling agents is referenced but poorly described in the literature (Bernetti et al., 1990; Southgate, 1991; Tsuge et al., 1990). The lack of information leads experimenters to use empirical approaches, which is responsible for a great variability in published results. It seemed necessary, due to this lack of data, to suggest a strategy allowing the determination of optimized dialysis conditions.

Dialysis parameters described in Table 2 were studied according to an experimental methodology based on the use of two fractional factorial designs. Membrane cutoff, water renewal (of dialysis bath), temperature, and dialysis time are controlled experimental factors. Starch, pectin, and sucrose concentrations are uncontrollable parameters associated with sample composition.

A first rank of 16 analyses, which were inferred from the experimental field (Table 2) and the structures of the designs, was achieved on test solutions S_1−S_8. Yields of sucrose elimination ($T\%$) varied between 41% and 93%.

The main effects (b_i) of factors (U_i) and the sums (b_{int}) of the first-order interaction effects (b_{ij}) were calculated by multilinear regression from previous results; they are presented in Figure 1. Each main effect (b_i) gave two types of information on each considered parameter. The (b_i) absolute value expresses the relative influence of the factor (U_i), while its sign shows the limit (U_i+) or (U_i−) of the field to consider for a better dialysis efficiency. The sums of interaction effects (b_{int}) described in Figure 1 are low, which means that first-order interaction effects (b_{ij}) are either negligible or large but with an opposite sign. The first hypothesis is the most likely. Under those conditions, the main effects (b_i) can be studied independently.

The effects b_1−b_4 correspond to the area of the experimental field (factors U_1−U_4) where it is possible to act to improve dialysis efficiency. Figure 1 highlights the preponderant influence of dialysis time (U_3) on the yield of sucrose elimination ($T\%$). It shows, in particular, that the choice of the limit U_3+ (24 h) significantly improves $T\%$.

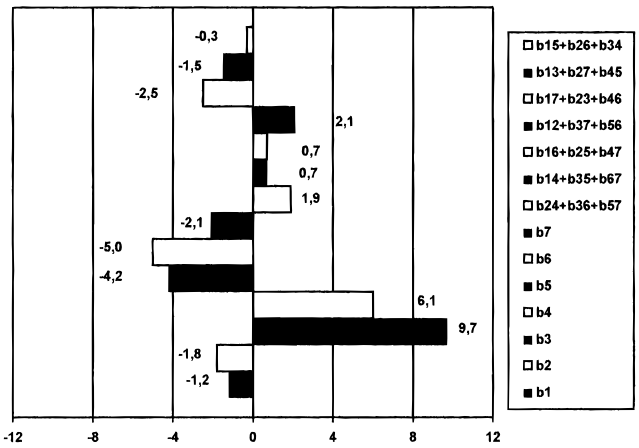

Figure 1. Graphic analysis of the effects (b_i and b_{int}) on the yield ($T\%$) of sucrose elimination.

U_4 is also an important parameter. A bath temperature of 35 °C (U_4+) contributes to the improvement of $T\%$ by reducing the viscosity of samples, which facilitates the solutes transfer toward the dialysis membrane. On the other hand, the effects b_1 and b_2, corresponding respectively to the type of membrane (U_1) and the water renewal flow of the dialysis bath (U_2), can be considered as negligible. Moreover, further tests have pointed out that it is possible to reduce U_2 to 128 mL/min without any yield ($T\%$) reduction. The effects of b_5-b_7 are linked to the samples and cannot be modified during the purification.

The pectin concentration (U_6) is the third factor in the order of influence after the time and the temperature of dialysis. Its representation in Figure 1 (b_6) shows that the dialysis yield ($T\%$) is higher with U_6- (pectin 0.3% w/w). This means that an increase of pectin concentration induces an increase of viscosity, which reduces dialysis efficiency. The similar effect of starch concentrations (U_5), however, less perceptible, than the pectin effect, is in agreement with this logical result.

The effect b_7 of sucrose concentration (U_7) can be considered as negligible. Its influence on $T\%$ is much lower than those of starch and pectin, the effect of its concentration on viscosity being less sensible. The dialysis of the test solution S_5 according to experimental conditions defined in this study (24 h, 35 °C, water renewal flow of 200 mL/min, cutoff 2000 Da) led to a yield ($T\%$) of 93% (residual sucrose concentration of 35 g/L). This result is the best yield of elimination obtained with the experimental designs. It validates the results of this study but reveals an insufficient improvement of $T\%$. Indeed, the concentration of glucose released from sucrose is still higher than the one released from starch. Thus, a further specific study on parameters of influence (time, temperature) was necessary.

Six time/temperature couples were applied to the simulation solution S5. The results showed a significant improvement of purification efficiency as time and temperature of dialysis increased. A yield ($T\%$) of 99.6% corresponding to a residual sucrose concentration of only 2 g/L was obtained by applying the 48 h/45 °C condition. Further analysis applying 72 h/45 °C led to a better purification but not to a total removal of sucrose. Forty-eight hours is therefore a reasonable compromise between dialysis efficiency and treatment time. Temperatures higher than 45 °C are not compatible with the membrane preservation. The dialysis conditions recommended from the second rank of analysis were checked on 15 SFP (SFP1−SFP15). Eighty-four analyses were carried out, leading to a mean yield of sucrose elimination ($T\%$) of 99.5% ($\sigma\% = 0.1\%$) and a residual glucose concentration from sucrose of about 1.5 g/L. This result is about 3 times lower than the lower concentration of glucose (5.4 g/L) from starch observed in SFP. The standard deviation ($\sigma\% = 0.1\%$) showed that the high viscosity of samples SFP13−SFP15, and the presence of fruit pieces in samples SFP1−SFP12, had no influence on the purification efficiency.

These results show that this method is suitable for the purification of a broad range of industrial SFP. They also demonstrate the efficiency of a methodological approach to optimize experimental conditions.

Study of Experimental Conditions of Gelatinization. The accurate determination by a single method of all modified starches used in SFP, independent of their chemical modifications, required a study of their gelatinization conditions. Blake and Coveney (1979) and Karkalas (1985) described this treatment as a simple way of improving the efficiency of enzymatic hydrolysis of modified starches. It provides, however, little information on the choice of experimental conditions depending on the type of starch modification. Interactions between starches modifications, pH value, and temperature of gelatinization were previously reported (Chatel et al., 1996). Four solutions (5.0 g/L) containing different modified starches (distarch phosphate, acetylated distarch phosphate, acetylated distarch adipate, and hydroxypropylated distarch phosphate) were submitted to different conditions of pH (neutral and alkaline) and temperature (20−80 °C) and then hydrolyzed and analyzed. In Figure 2, curves show the major influence of the pH of gelatinization on starch determination. Starch concentrations determined in samples gelatinized in alkaline media were always higher than or equal to those obtained after neutral treatment. Those concentrations were almost identical to the initial one (5.0 g/L) for distarch phosphate, acetylated distarch phosphate, and acetylated distarch adipate. These results demonstrate that the alkali dispersion of those three starches improves their quantification.

Figure 2 shows also the weak influence of temperature on starch quantification in alkalinized solutions of distarch phosphate, acetylated distarch phosphate, and acetylated distarch adipate. In neutral medium, the concentrations of acetylated distarch phosphate and acetylated distarch adipate increased with temperature, to reach a maximum of 4 g/L above 60 °C. That concentration remains lower than the one obtained at 20 °C in alkaline medium. The same value was obtained in both media at 70 °C for the distarch phosphate, due to the weak resistance to hydrolysis of nonacetylated starches. Figure 2 reveals the specificity of hydroxypropylated distarch phosphate, which is not influenced by temperature and pH conditions. Hydroxypropyl groups confer on this starch a strong resistance against hydrolysis since the ether bonds between those groups and glucoside residues are not sensitive to the gelatinization conditions. The determined yield for this starch was about 60%, which is in accordance with literature data (Blake and Coveney, 1979; Karkalas, 1985). The improvement of experimental conditions of gelatinization (70 °C, 30 min, alkaline medium) allowed the determination of acetylated distarch phosphate and acetylated distarch adipate concentrations with the same accuracy and roughly the same yield as for

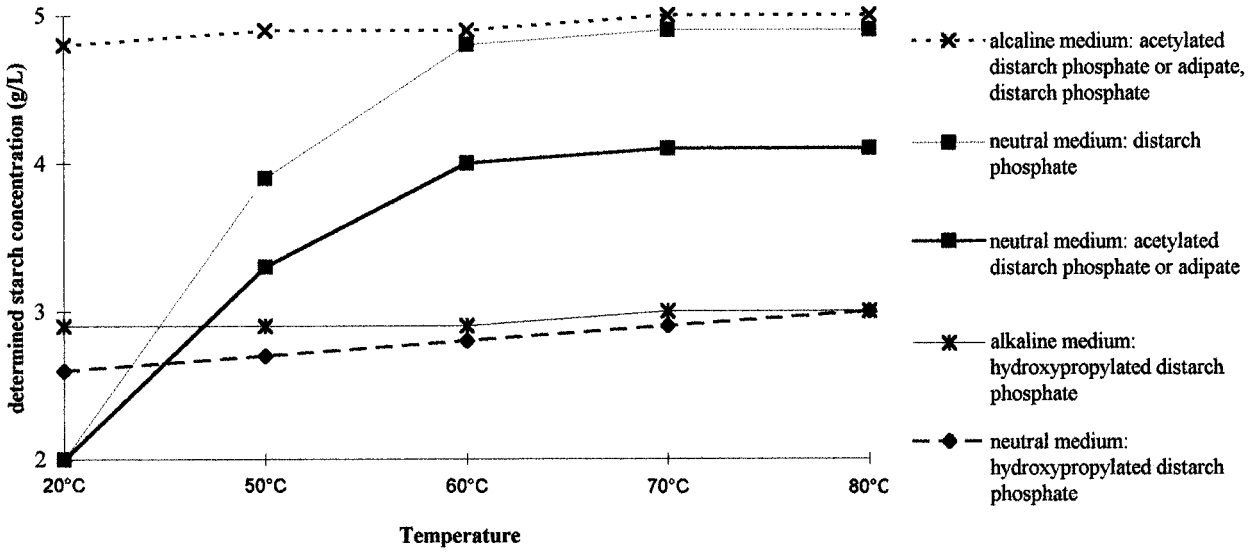

Figure 2. Influence of gelatinization conditions on modified starches determination. Experiments were realized on 5.0 g/L starch solutions.

distarch phosphate. These results confirm the standardization effect of gelatinization for the different modified starches, except for hydroxypropylated starch, which requires a specific treatment.

Identification of Modified Starches by FT-IR Spectroscopy. Published results (Blake and Coveney, 1979; Chatel et al., 1996; Karkalas, 1985) and our results showed that hydroxypropylated distarch phosphate quantification is underestimated because of its resistance to hydrolysis. The difference between this modified starch and the others justifies the necessity of identification of the chemical modifications, before quantification.

Several identification techniques are described in the literature. The most relevant are gas chromatography for distarch adipate (Mitchel et al., 1982), infrared for acetylated starches (Van der Bij and Vogel, 1962), and RMN and FT-IR for hydroxypropylated starches (Forrest, 1992). This last method seems to be adaptable to the analysis of the modified starches used in SFP. It is a simple technique without long sample preparation, which permits the identification of acetylated and hydroxypropylated starches from the same spectrum scanning.

A first rank of experiments achieved on 15 SFP (SFP1−SFP15) has demonstrated that the extracted starches spectra were identical to those of pure modified starches.

The identification of acetylated starches (Figure 3) was made by comparing SFP2 (distarch phosphate, 3% w/w) and SFP4 (acetylated distarch phosphate, 3% w/w) spectra. The only difference was an additional band on the SFP4 spectrum at 1724 cm^{-1} corresponding to the stretching vibration of the C═O bond from the acetyl group (Van der Bij and Vogel, 1962). This band was observed in the spectra of all SFP containing acetylated starches (SFP3−SFP8, SFP13−SFP15) but was absent in spectra of samples containing distarch phosphate (SFP1, SFP2) or hydroxypropylated distarch phosphate (SFP9−SFP12). The identification of acetylated starches in SFP can be achieved owing to this distinctive band at 1724 cm^{-1}. No significant difference was observed between the spectra of SFP2 (distarch phosphate, 3% w/w) and SFP12 (hydroxypropylated distarch phosphate, 3% w/w). On the other hand, their second

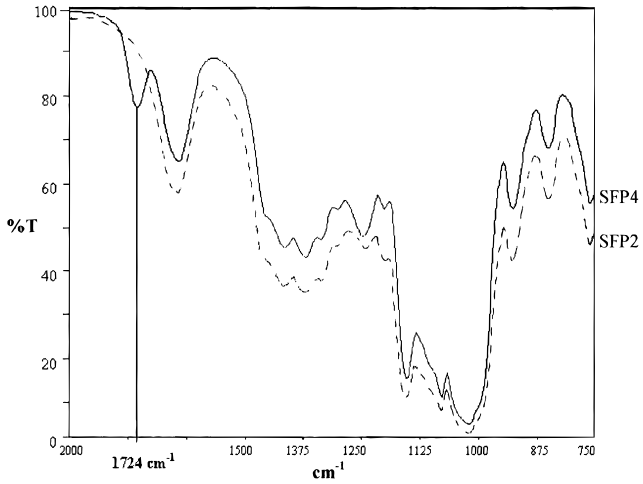

Figure 3. FT-IR identification of acetylated starches (2000−750 cm^{-1}).

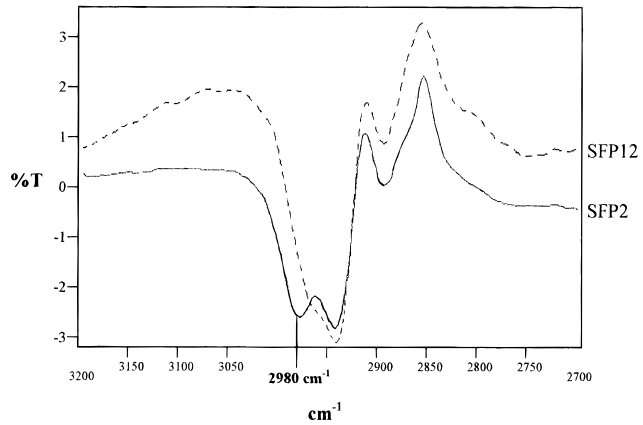

Figure 4. FT-IR identification of hydroxypropylated starches (second derivatives 3200−2700 cm^{-1}).

derivatives between 3200 and 2700 cm^{-1} (Figure 4) allowed the identification of an additional band at 2980 cm^{-1} for SFP12, which corresponds to the stretching vibration of the C−H bond of the hydroxypropyl group methyl function (Forrest, 1992). This band was ob-

Table 3. Modified Starches Determination in SFP

sample	starch concn[a] (g/100 g)	starch determination[a] (g/100 g)	SD[b] (σ%)	no. of expts
distarch phosphate				
SFP1	0.50	0.45	4.2	12
SFP2	3.00	2.80	2.6	10
acetylated distarch phosphate				
SFP3	0.50	0.43	4.4	12
SFP4	3.00	2.77	4.2	11
acetylated distarch adipate				
SFP5	0.50	0.40	4.3	15
SFP6	1.00	0.84	4.1	11
SFP7	2.00	1.84	4.1	12
SFP8	3.00	2.74	3.5	15
SFP13	2.90	2.66	1.3	8
SFP14	2.90	2.65	2.1	8
SFP15	2.90	2.66	1.3	8
hydroxypropylated distarch phosphate				
SFP9	0.50	0.22	4.4	12
SFP10	1.00	0.44	3.0	12
SFP11	2.00	1.00	6.0	12
SFP12	3.00	1.55	5.4	12

[a] Results expressed on wet basis: moisture content 13% (w/w). [b] Relative standard deviation.

served for all SFP containing hydroxypropylated starches (SFP9−SFP12) and was absent in samples containing all other starches (SFP1−SFP8, SP13−SFP15). This band can be considered as characteristic of hydroxypropylated starches in SFP.

FT-IR does not distinguish the acetylated distarch adipate from the acetylated distarch phosphate. The different types of reticulation, adipate or phosphate, cannot be pointed out by comparison between spectra of SFP4 (acetylated distarch adipate, 3% w/w) and SFP8 (acetylated distarch phosphate, 3% w/w). However, this has no influence on the determination of starches concentrations since these modifications, unlike hydroxypropylation, do not affect the accuracy.

Statistical Study of Modified Starches Determination in SFP. One hundred and seventy experiments were carried out on 15 different samples (SFP1−SFP15) to validate the proposed method for the determination of modified starches concentrations in SFP.

Similar results close to real concentrations were obtained for distarch phosphate, acetylated distarch phosphate, and acetylated distarch adipate (see Table 3). It is the direct consequence of the improvement of gelatinization conditions which permitted the standardization of the hydrolysis yield of the modified starches studied. Hydroxypropylated distarch phosphate was determined to be around 50% of the real concentration in samples SFP9−SFP12. It is in agreement with literature values (Karkalas, 1985) but dramatically lower than the 80−93% reached with other modified starches.

Relative standard deviations (σ%) between 2.6 and 4.4% were obtained (Table 3) for samples SFP1−SFP8. This demonstrates that for distarch phosphate, acetylated distarch phosphate, and acetylated distarch adipate, the proposed determination method is reproducible for the whole range of concentrations from 0.5% to 3.0% (w/w). Moreover, it was shown from samples SFP13−SFP15 that the type of fruit (apricot for SFP15) and the presence of guar (SFP13−SFP15) or xanthan (SFP13) had no influence on the determination accuracy. It can also be observed that the relative standard deviations are smaller (1.3−2.1% instead of 3.5−4.3%) for the samples (SFP5−SFP8) without fruit pieces. The higher standard deviations (σ% = 3−6%) were obtained for hydroxypropylated distarch phosphate, as expected, but they were still reasonable. The method is less sensitive to the chemical modifications of starches than to sucrose interferences due to unsufficient dialysis.

The restricted gap between real and experimental concentrations (except for hydroxypropylated starch) and the good reproducibility of the method allow the definition of correction coefficients. In Table 4, a linear relationship between real and experimental concentrations is presented for each starch studied.

Identical weak slopes (1.06) and low intercept values (0.03−0.08 g/100 g) were obtained with distarch phosphate, acetylated distarch phosphate, and acetylated distarch adipate. This shows that only limited corrections are necessary for those three starches. It also demonstrates that the absence of distinction between adipate and phosphate distarches has a weak influence on their quantitative determination in unknown samples.

Higher slope (1.86) and intercept values were calculated for hydroxypropylated distarch phosphate, which shows that a larger correction is required for this starch and confirms the relevant of the FT-IR identification. The quality of the linear regressions was judged on the basis of correlation coefficients (Table 4).

It is essential to notice that the validity of the proposed equations should be verified before the analysis of starches other than the ones studied here.

Conclusion. A complete method for the identification and the determination of modified starches concentrations in SFP was suggested. The first step is an optical microscopy observation of the sample to exhibit starch granules. An extensive identification of chemical modifications by FT-IR allows the selection of a suitable correction coefficient. This method does not distinguish the acetylated distarch phosphate from the acetylated distarch adipate. This phenomenon has no influence on the determination of starches concentrations since these modifications do not affect the accuracy.

The main step of sample preparation is their purification by dialysis to eliminate the greater part (>99%) of sucrose. The gelatinization step in an alkaline medium allows the standardization of several modified starches toward hydrolysis by amylolytic enzymes such as α-amylase and amyloglucosidase. Finally, the released glucose concentration can be accurately determined with excellent reproducibility owing to the hexokinase/glucose-6-phosphate dehydrogenase system.

Table 4. Linear Regressions and Correlation Coefficients

modified starch	linear regression	correl coeff
distarch phosphate	$[\text{starch}]_{\text{real}} = 1.06[\text{starch}]_{\text{exp}} + 0.03$	0.99907 ($n = 22$)[a]
acetylated distarch phosphate	$[\text{starch}]_{\text{real}} = 1.06[\text{starch}]_{\text{exp}} + 0.05$	0.99722 ($n = 23$)[a]
acetylated distarch adipate	$[\text{starch}]_{\text{real}} = 1.06[\text{starch}]_{\text{exp}} + 0.08$	0.99756 ($n = 53$)[a]
hydroxypropylated distarch phosphate	$[\text{starch}]_{\text{real}} = 1.85[\text{starch}]_{\text{exp}} + 0.15$	0.99462 ($n = 48$)[a]

[a] n, number of experiments.

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