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DSC as a tool to assess physiological evolution of apples preserved by edibles coatings

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Abstract

The present study describes the results obtained on the evolution of apples preserved by edible coatings (alginate and gelatine based) by using differential scanning calorimetry (DSC) as an analytical tool, and their comparison to the textural evolution was measured by conventional methods and carbohydrate analysis by HPLC. The evolution of thermal transitions obtained from lyophilized apple fractions obtained from peel, pulp and core was monitored. Differences observed in the thermograms caused by the application of coatings to the apples are also studied. DSC results when compared to those of textural analysis seem to produce earlier responses to the physiological changes in apples. When edible coatings were applied, DSC profiles were found to be quite different from those of uncoated samples: two transitions are found in uncoated apples and three or four in the coated ones. As it was expected, both coatings affected the apples physiological evolution, but in dissimilar way. The tested methodologies are effective to monitor those post harvest changes. However, DSC analysis may be useful as a quicker and efficient method, eventually detecting changes earlier than conventional methodologies.

Keywords: DSC; Apple; Edible coating; Preservation; HPLC

1. Introduction

The extension of fruit shelf life is an important goal to be attained. Many storage techniques have been developed to extend the marketing distances and holding periods for commodities after harvest. Different preservation methodologies have been studied. One method of extending post harvest shelf life is the use of the edible coatings (Baldwin, Niesperos, Shaw, & Burns, 1995). Such coatings are used to enrobe fresh produce, providing a semi permeable barrier and create an internal modified atmosphere delaying ripening and reducing decay (Baldwin et al., 1995; Baldwin et al., 1999; Kester & Fennema, 1986; Nisperos-Carriedo, 1994). The modification of internal gas composition is achieved through the regulation of moisture, oxygen, carbon dioxide, lipid, aroma and flavour compounds transfer in food systems (Baldwin, Niesperos-Carriedo, Hagenmaier, & Baker, 1997; McHugh, Huxsoll, & Krochta, 1996). However, a certain degree of oxygen and carbon dioxide permeability is necessary to avoid anaerobiosis, which would result in physiological disorders and a rapid loss of quality. These goals may be attained through the use of coatings (Baldwin et al., 1995). Some studies are found in the literature concerning the use of edible coatings in apples preservation (Bai, Hagenmaier, & Baldwin, 2003; Moldão-Martins, Beirão-da-Costa, & Beirão-da-Costa, 2003; Saftner, Conway, & Sams, 1998; Sümnü & Bayındırlı, 1995). The performance of coatings on apples preservation was related to their O_2 and CO_2 permeabilities, with higher permeability resulting in lower internal CO_2 and higher internal O_2. The excessive modification of internal gas induced an abrupt rise of the fruits respiratory quotient, prodigious accumulation of ethanol in 'Braeburn' and 'Granny Smith' (Bai et al., 2003).

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The functional characteristics required for a coating depends on the product matrix and on the deterioration processes the product is subject to. Potential applications and properties of edible films and coatings have been reviewed elsewhere (Guilbert, Gontard, & Gorris, 1996; Kester & Fennema, 1986). Proteins, lipids and polysaccharides are the main constituents of edible films and coatings. Among the studied proteins are wheat gluten, corn zein, soy protein rice protein, egg albumin, milk proteins and gelatine (Sobral, Menagalli, Hubinger, & Roques, 2001). In this respect, lipids also include waxes, acylglycerols and fatty acids. Suitable polysaccharides include cellulose and derivatives, alginates, pectins, starch and derivatives and others (Sobral et al., 2001). Sucrose polyesters at different concentrations were effective for extending the shelf life of apples (Chai, Ott, & Cash, 1991).

The shelf life can be predicted by either controlling the driving agents or monitoring their effects. Although the analytical methods generally used to detect quality changes can nowadays be well refined, they only provide indirect information about the transformation mechanisms and may be related to effects (e.g. acidification sustained by the microbial growth) which are delayed with respect to the main degradation process (Riva, Fessas, & Schiraldy, 2001).

Foods are thermodynamically metastable systems, as their properties change over the time depending on chemical composition, enzymatic activity and storage conditions. Some studies (Riva et al., 2001) support the idea of the reliability of calorimetric monitoring, mainly differential scanning calorimetry (DSC), to evaluate the shelf life of foods. As stated by Aguilera, Cuadros, and del Valle (1998) referring Slade and Levine (1991) and Roos (1995), there is considerable interest in studying thermal transition in food polymers, particularly to test the hypothesis that there is a drastic decrease in mechanical and viscoelastic properties and increased molecular mobility in the food matrix at temperatures above T_g. A comparison between the results of traditional techniques and the calorimetric monitoring supported the reliability of the latter, which offers some peculiar advantages, like better temperature control, continuous follow-up, easier mathematical description, and overall energy balance of the degradation process (Riva et al., 2001).

Aguilera et al. (1998) applied DSC to detect thermal phase transitions (T_g) in low moisture apple products and cell walls. Besides, the authors also found an endothermic peak between 50 and 70 °C in freeze dried apple probably corresponding to soluble polysaccharides.

In general, references found in the literature are mainly related to glass transition studies, and so the samples always contain a certain amount of water. The references are scarce about studies on other transition phenomena conducted on samples after drying.

The aim of the present study was (i) to evaluate the applicability of differential scanning calorimetry to the study of apples evolution, coated and uncoated, along preservation period and (ii) to perceive eventual different evolution patterns among them.

2. Materials and methods

2.1. Materials

"Golden Delicious" apples were selected for their uniformity in size (Ø65–70 mm) and freedom from defects. Raw material was characterized for external colour ($L = 80.8$; $C = 24.1$; $h = 181.39$) and firmness (9.5 N).

2.2. Edible coatings preparation

Two edible coatings were used: based in alginate (SATIALGINE S550 Laboratórios SANOFI Bio-Industries) and gelatine (commercial powder).

The coatings were prepared using (i) 2% alginate + 1% glycerol (w/w), (ii) 5% gelatine + 1% glycerol (w/w). The components were homogenized by magnetic stirring at 45 °C.

2.3. Edible coatings application

The coatings were applied by dipping the apples for 15 s. After dipping in the coating, the fruits were drained at room conditions. The controls (uncoated apples) were immersed in distilled water under similar conditions.

Coated and uncoated fruits were held for three months at refrigerated conditions (4 °C).

2.4. Methods

2.4.1. DSC analysis

DSC analysis was performed in a Shimadzu DSC 50 equipped with a TA 50 SI thermal analyser. Helium (99.95% purity) was the purge gas and flowed at approximately 20 mL min^{-1}. The calorimeter was calibrated according to a standard procedure established in the manufacturer user's manual. The DSC instrument was calibrated using indium (m.p.: 156.6 °C, $\Delta H_f = 28.45$ J g^{-1}) and deionised water (m.p.: 0 °C, ΔH_m: 333 J g^{-1}). The calibration procedure was completed with the scanning rate to be used in the trials.

For DSC analysis apples fractions – peel, pulp and core – were produced and lyophilized.

Samples of 7.0 ± 0.1 mg were weighed into aluminium pans (inner volume: ca. 30 μL), and covers were hermetically sealed into place. An empty, hermetically sealed aluminium pan was used as the reference. Prior to the analysis of samples, the baseline was defined with an empty, hermetically sealed aluminium pan. The samples were subjected to the following heating program: from 20 °C to 25 °C at a rate of 10 °C/min, holding for 3 min at 25° for stabilizing, and from 25 °C to 300 °C at a rate of 7.5 °C/min.

Thermal transitions were defined in terms of peak or transition temperature (T_n). Heat of transition or enthalpy ΔH ($J\,g^{-1}$) was evaluated from peak areas and results expressed per weight (g) of sample. The manufacturer's software program was used to analyse and plot the thermal data (Anon, 1995).

In order to make possible the identification of compounds in the thermograms, DSC analysis was previously applied to mono, di, oligo and polysaccharides as well as to coating ingredients and prepared coatings.

2.4.2. Texture

For texture analysis a texturometer TA-XT2 was used, using a cylindrical probe of 2 mm diameter. A compression test to attain 7 mm deformation at 1.5 mm/s rate was performed.

2.4.3. Colour

External fruit colour was measured by the L*a*b* system using a chroma meter (Minolta Colour Meter CR 200). A white tile (L^*: 97.46; a^*: −0.02; b^*: 1.72) was used as reference.

2.4.4. Sugar composition

Main sugars and sorbitol and butanol were evaluated by HPLC system consisting of a 125 Beckman pump, a differential refractometer model 401, a R460 Waters detector and a Waters 745 integrator. A sugar-pack 1 column from Waters was used. The mobile phase consisted of EDTA-Ca 50 ppm solution, at the flow rate of 0.5 ml/min and 90 °C. The sample volume injected was 20 μL. Detection was made by refraction index, PI sorbitol.

3. Results and discussion

Apples are a complex matrix in which carbohydrates are the main constitutive compounds and thereby probably those that better translate physiological activity. In order to better understand the apples physiological evolution, in a first trial, thermal transitions of several carbohydrates, coating ingredients and prepared coatings, were established, identifying the involved compounds and the possible interferences of the coatings. Table 1 shows the obtained results for transition temperatures of the reference materials. It can be concluded about that the present results are in accordance to the results of the other authors, namely Roos (1993).

With the aim to evaluate if any apple fraction is more sensitive than the others to DSC transitions, thermograms of different fractions (peel, pulp and pit) of uncoated apples were performed. DSC profiles (Fig. 1) always presented two endotherms (T_1 and T_2 at about 135 °C and 195 °C, respectively). Having in mind that the samples are lyophilised those transitions may be mainly related to the presence of the fructose and sucrose, respectively. However, peel presents a broader range of temperatures for the first transition, to which other compounds such as the main constituent of pectic substances, i.e. galacturonic acid may contribute.

Table 1
Melting temperatures for several reference compounds and coatings as determined by DSC

Compound	T_1 (°C)	T_2 [°C]	T_m (Roos, 1993) (°C)
L-Arabinose	160	–	–
D-Arabinose	–	–	160
D-Ribose	–	–	86
D-Xylose	157	–	157
D-Fructose	126	–	127
D-Glucose			158
D-Glucose · H_2O	83	–	86[a]
Sucrose	193	–	190
Cellobiose	244	–	–
Raffinose × $5H_2O$	–	–	80
D-Sorbitol	–	–	99
D-Mannitol			167
Xylitol			95
Maltitol			149
Galacturonic acid	114	–	–
Glycerol	117	222	–
High methoxyl pectin	150	–	–
Cellulose	116	–	–
Gelatine[b]	83	107	–
Alginate	135	170	–
Gelatine coating[c]	81	238	–
Alginate coating[c]	127	207	–

[a] Weast, 1986.
[b] Denaturation.
[c] Melting/Degradation.

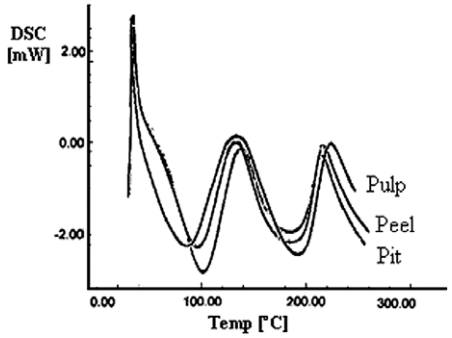

Fig. 1. DSC profiles of uncoated fruit fractions (peel, pulp and pit).

Enthalpy changes were also used on thermogram analysis. Enthalpy variations are observed for both transitions along time. The three fruit regions showed similar profile patterns, being only different in the intensity of the changes. Fig. 2 presents the textural evolution and enthalpy variations of the apples along time. There seems to exist some relationship between enthalpies variation pattern and texture evolution profile. A significant increase in enthalpy values during first storage stage (two weeks) can be observed, this increase being higher for E1 of the peel (variation of about 55 J/g). This change in enthalpy values may be related to the breakdown of pectic substances. During the same period, a significant decrease in texture, that is

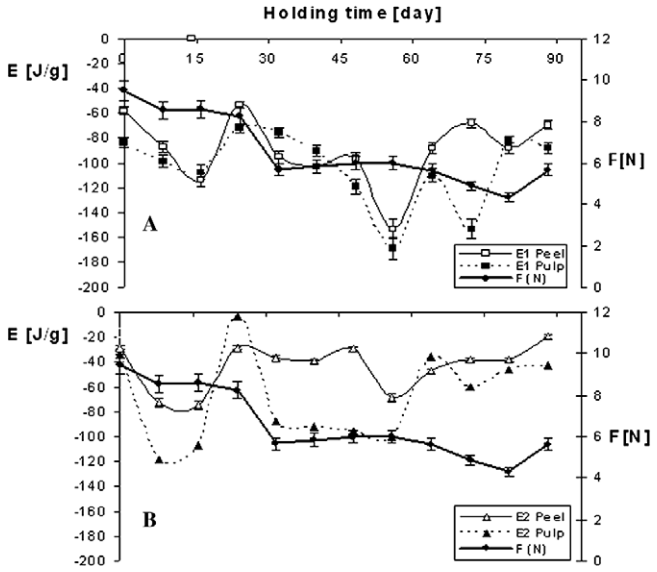

Fig. 2. Firmness evolution profile (F) of uncoated fruits and enthalpy variations observed for endotherm 1 (E1) and endotherm 2 (E2) along the holding time. A – Peel, B – pulp.

only observed after one month (3 N), was not observed. In this respect, a decrease in the enthalpy values was observed in the 3rd week, mainly on E2 of the pulp (103 J/g). This effect may probably be due to an increment on sugar consumption (a decrease of about 4 g/100g both in sucrose and fructose content), expressing an increase in the respiratory rate accompanied by texture softening.

After one month of storage, changes in firmness are not instrumentally detected due to the higher peel elasticity. However, after this period a noticeable decay was sensorially detected (results not shown). By DSC analysis a significant increase in enthalpy values was observed in the same period, probably due to carbohydrate hydrolysis. Further studies are needed in order to better understand the relationship between DSC results, physiological evolution and texture decrease.

Analysing the evolution pattern of both carbohydrates as evaluated by HPLC (Table 2) and detected by DSC analysis (Table 3), it can be stated that sucrose may be identified with the second transition along all the holding period. The observed decrease in sucrose content is detected by both analysis methodologies and translated in the thermograms by lower enthalpy values. The first transition is not always related to the same compound. Most of the time, the first transition is related to fructose but in the first phases of the storage period it seems to be related to compounds showing lower temperature transition like ribose, raffinose and galacturonic acid. However, HPLC analysis did not detect significant differences either in raffinose or in fructose content along storage. So, this fact may be eventually explained by the form in which compounds are found along storage or by changes in ribose content not analysed by HPLC. The results found for peel, pulp and pit are similar, except in the case of the pit where pectin was identified. In face of these results, it may be assumed that DSC analysis presents better sensitivity to show relevant differences encountered along the holding period.

In the case of the coated fruits, by thermal analysis three or four endotherms were detected, as is exemplified in Fig. 3 representing a thermogram of the pulp of gelatine coated apple. It is noticed that along the holding period, the transitions are not always observed at the same temper-

Table 2
Carbohydrates content (g/100g) as analysed by HPLC for uncoated fruits, coated with alginate and gelatine in pulp along the holding period

Holding time [day]	0	30	45	60	75	90
Uncoated						
Raffinose	0.08 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.06 ± 0.01	0.12 ± 0.01	0.15 ± 0.01
Sucrose	14.35 ± 0.72	15.35 ± 0.77	12.92 ± 0.65	7.99 ± 0.40	5.50 ± 0.27	6.73 ± 0.34
Glucose	15.51 ± 0.78	8.92 ± 0.45	13.64 ± 0.68	12.51 ± 0.63	18.50 ± 0.93	14.51 ± 0.73
Fructose	66.45 ± 3.32	63.80 ± 3.19	65.49 ± 3.27	68.25 ± 3.41	66.75 ± 3.34	29.08 ± 1.45
Sorbitol	2.18 ± 0.11	2.18 ± 0.11	3.03 ± 0.15	2.49 ± 0.12	3.00 ± 0.15	2.82 ± 0.14
1-Butanol				0.39 ± 0.01		
Alginate coated						
Raffinose	0.08 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.08 ± 0.01	0.14 ± 0.01	0.11 ± 0.01
Sucrose	14.35 ± 0.72	6.60 ± 0.33	7.84 ± 0.39	2.03 ± 0.10	2.86 ± 0.14	1.42 ± 0.07
Glucose	15.51 ± 0.78	14.95 ± 0.75	17.31 ± 0.87	16.14 ± 0.81	21.28 ± 1.06	16.72 ± 0.84
Fructose	66.45 ± 3.32	68.33 ± 3.42	66.25 ± 3.31	78.77 ± 3.94	62.97 ± 3.15	54.90 ± 2.75
Sorbitol	2.18 ± 0.11	4.54 ± 0.23	3.78 ± 0.19	6.19 ± 0.31	6.59 ± 0.33	7.43 ± 0.37
1-Butanol				0.32 ± 0.02	1.08 ± 0.05	2.28 ± 0.11
Gelatine coated						
Raffinose	0.08 ± 0.01	0.10 ± 0.01		0.11 ± 0.01	0.19 ± 0.01	0.08 ± 0.01
Sucrose	14.35 ± 0.72	8.55 ± 0.43	3.01 ± 0.15	6.79 ± 0.34	5.34 ± 0.27	2.95 ± 0.15
Glucose	15.51 ± 0.78	15.39 ± 0.77	20.80 ± 1.04	13.18 ± 0.66	14.02 ± 0.70	17.79 ± 0.89
Fructose	66.45 ± 3.32	61.83 ± 3.09	65.92 ± 3.30	60.00 ± 3.00	63.52 ± 3.18	67.13 ± 3.36
Sorbitol	2.18 ± 0.11	4.05 ± 0.20	3.54 ± 0.18	4.31 ± 0.22	5.76 ± 0.29	5.53 ± 0.28
1-Butanol				0.69 ± 0.03		

Table 3
Carbohydrates identification by DSC analysis sequences for uncoated fruits (UC), coated with gelatine (GC) and alginate (AC) in pulp and pit fractions

	Day	Pulp			Pit			
		T1	T2	T3	T1	T2	T3	T4
UC	0	(Raffinose/ribose)?	Sucrose		(Raffinose/ribose)?	Sucrose		
	30	(Raffinose/ribose)?	Sucrose		Fructose	Sucrose	Pectin	
	45	Fructose	Sucrose		Fructose	Sucrose		
	60	Fructose	Sucrose		Fructose	Sucrose		
	75		Sucrose		Fructose	Sucrose		
	90		Sucrose		Fructose	Sucrose		
GC	0	(Raffinose/ribose)?	Sucrose	Mannitol?	(Raffinose/ribose)?	Sucrose		n.i.
	30	Fructose	Sucrose	Mannitol?	(Raffinose/ribose)?	Sucrose		n.i.
	45		Sucrose	Mannitol?	Fructose	Sucrose		n.i.
	60		Sucrose	Mannitol?		Sucrose	Pectin	n.i.
	75	Fructose	Sucrose	Mannitol?		Sucrose		n.i.
	90	Fructose	Sucrose	Mannitol?		Sucrose		n.i.
AC	0	(Raffinose/ribose)?	Mannitol?	n.i.	(Raffinose/Ribose)?	Mannitol?		n.i.
	30	(Raffinose/ribose)?	Mannitol?	n.i.	(Raffinose/ribose)?		Pectin	n.i.
	45	(Raffinose/ribose)?	Mannitol?	n.i.	Sorbitol		Pectin	n.i.
	60	(Raffinose/ribose)?	Mannitol?	n.i.		Mannitol?	Pectin	n.i.
	75	Sorbitol	Mannitol?	n.i.				n.i.
	90		Mannitol?	n.i.				

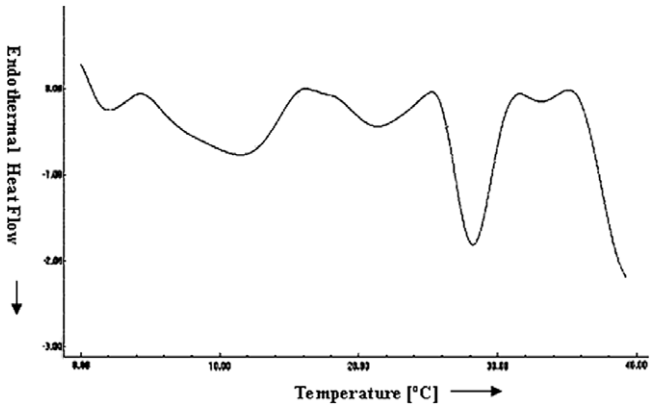

Fig. 3. Pulp representative DSC profile of a gelatine coated apple.

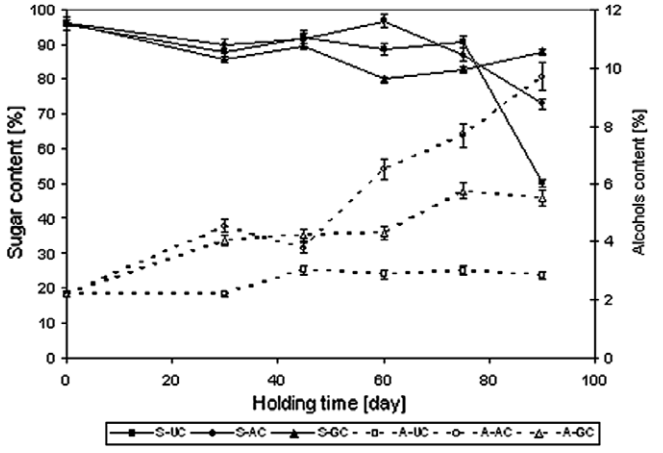

Fig. 4. Total main sugars (sucrose + fructose + glucose) (S) and alcohols (sorbitol + butanol) (A) evolution on the pulp of uncoated (UC), gelatine (GC) and alginate (AC) coated fruits.

atures, this being due to the presence of different compounds.

In the case of alginate coated fruits, first transition was never attributed to fructose in opposition to what happens in uncoated fruits. On uncoated fruits, compounds presenting low melting temperatures are only present till the 30th day. On alginate coated fruits, the compounds are preserved for a longer period (60th day) and then compounds showing higher melting temperature than fructose are detected. The second transition is either associated to sucrose melting or eventually to another kind of compounds (mannitol). HPLC analysis shows a decrease in main sugars (sucrose + glucose + fructose) content during the first 30 days for both coated and uncoated samples (Fig. 4). Afterwards different evolution patterns were found among the samples. At the end of the storage period, sugar content in uncoated samples is much lower than in coated samples. The observed decrease in fructose content between 75 and 90 days on uncoated fruits is a clear sign of the higher metabolic activity registered on these fruits. Meanwhile, the alcohols content that remains almost constant on the uncoated samples suffers a significant increase on coated samples, being higher on alginate coated samples. This effect is more pronounced after the 45th day. The third and fourth endotherms seem to be related to pectin, cellobiose or to nonidentified compounds showing higher melting temperatures. The presence of pectin, only detected on uncoated fruits on 30th day, is identified in alginate coated fruits till the 60th day, showing a lower enzymatic activity. On the other hand, sugar alcohols like sorbitol and alcohols (butanol) significantly increase. This aspect may be explained probably by coating a tighter matrix, making difficult the gaseous transfer phenomena and so leading to more anaerobic conditions or to metabolization of fatty compounds contained in the coating (Oli-

vas, Mattinson, & Barbosa-Cánovas, 2005) or to production of the aromatic compounds. The existence of a tighter matrix coating in the case of the alginate may be supported by the DSC profile of both gelatine and alginate and produced coatings. In fact, as can be seen on Table 1 higher values were found for the thermal transitions of alginate and alginate coated than for gelatine, indicating a stronger network.

In conclusion, there seems to exist some relationship between enthalpies variation pattern and texture evolution profile; however, further studies are needed in order to better understand the relationship between DSC results, physiological evolution and texture decrease. As it was expected, both coatings affected the apples physiological evolution, but in a dissimilar way. The tested methodologies are effective to monitor those post harvest changes. However, DSC analysis may be useful as a quicker and efficient method, eventually detecting changes earlier than conventional methodologies.

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