Research and Characterization of New Materials for the Production of Edible Coatings

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Abstract

Tropical fruits are subjected to great losses from harvest to consumption. The main objective of this work was to research and characterize new materials as galactomannans and determine the optimal composition of galactomannan-based coatings in view of their application to extend the shelf life of several tropical fruits. Galactomannans extracted from seeds of *Caesalpinea pulcherrima*, *Adenanthera pavonina*, *Gleditsia triacanthos* and *Sophora japonica* were characterized as coatings for five tropical fruits: acerola (*Malpighia emarginata*), cajá (*Spondias lutea*), mango (*Mangifera indica*), pitanga (*Eugenia uniflora*) and seriguela (*Spondias purpurea*). The intrinsic viscosity of galactomannans solutions with and without glycerol, were determined. The surface properties of fruits was determined, and different formulations of aqueous galactomannan solutions (0.5 %, 1.0 % and 1.5 %) with glycerol (1.0 %; 1.5 % and 2.0 %) were tested in terms of wettability on the five fruits. To analyse the capacity of the galactomannans to decrease the fungal growth, tests using four strains of fungi were performed with: *Penicillium commune*, *Penicillium crustosum*, *Penicillium roqueforti* and *Botrytis cinerea*. The galactomannan that demonstrated to provide a more significant fungal inhibition was *G. triacanthos*. The *C. pulcherrima* seed galactomannan was analysed to determine its sub-chronic toxicity in mice, measuring the evolution of weight, glycemic levels and total cholesterol levels during three months; no toxicity was observed. The weight loss from acerola and mango, coated with galactomannan from *C. pulcherrima,* during storage time was determined, giving good indications that the use of solutions of this galactomannan and glycerol as coating decreases the weight loss of the fruits. The obtained results confirmed the suitability of galactomannan-based coatings to prolong the shelf life of tropical fruits.

Resumo

Os frutos tropicais são sujeitos a grandes perdas desde a colheita até o consumo. O objectivo principal deste trabalho foi determinar a composição óptima dos revestimentos de galactomananas, tendo como finalidade aumentar o *shelf life* de frutos tropicais. Galactomananas extraídas de sementes de *Caesalpinea pulcherrima*, *Adenanthera pavonina, Gleditsia triacanthos* e *Sophora japonica* foram solubilizadas e caracterizadas como revestimento de cinco frutos tropicais: acerola (*Malpighia emarginata*), cajá (*Spondias lutea*), manga (*Mangifera indica*), pitanga (*Eugenia uniflora*) e seriguela (*Spondias purpurea*). A viscosidade intrinseca foi determinada com soluções de galactomananas com e sem glicerol. As propriedades de superfície dos cinco frutos foram determinadas, e diferentes formulações de soluções aquosas de galactomanana (0.5 %, 1.0 % e 1.5 %) com glicerol (1.0 %; 1.5 % e 2.0 %) foram testadas em termos de *wettability* nos cinco frutos. Com o objectivo de analisar a capacidade das galactomananas estudadas em decrescer o crescimento

fúngico, foram realizados testes com cinco estirpes de fungos: *Penicillium commune*, *Penicillium crustosum*, *Penicillium roqueforti* e *Botrytis cinerea*. A galactomanana que demonstrou um maior efeito na inibição no crescimento fúngico foi a *G. triacanthos*. A galactomanana de *C. pulcherrima* foi usada para determinar a toxicidade sub-crónica, avaliando a evolução dos pesos, colesterol e níveis de glicemia de ratinhos durante 3 meses. Não foi observada toxicidade para a galactomanana de *C. pulcherrima* quando comparada com o grupo de controlo. Também se avaliou a perda de massa da acerola e da manga durante o seu armazenamento, demonstrando-se que os revestimentos de *C. pulcherrima* diminuem a perda de massa dos frutos. Os resultados obtidos confirmaram a possibilidade de aplicar os revestimentos de galactomananas em frutos tropicais para lhes aumentar o tempo de prateleira.

Introduction

During the last decades, there has been an increase in the demand for fresh fruit and vegetable products that forced the industry to develop new and improved methods for maintaining food quality and extending shelf life. Great losses (from 20 to 80%) in the quality of fresh fruits occur between their harvest and consumption, and one of the most important drawbacks in fruit distribution chains is their short shelf life (1). On the other hand, consumers around the world demand food of high-quality, without chemical preservatives, and with extended shelf life. So, an increased effort has been made to discover new natural preservatives and antimicrobials (2). The main factors responsible for extending the shelf life of fruits and vegetables include: careful harvesting (as not to injure the product), harvesting at optimal horticultural maturity and good sanitation (3, 4). When these practices are applied, the implementation of optimum storage conditions through modified atmosphere can be quite effective at maximizing the shelf life and quality of the product. This is done by controlling factors such as temperature, relative humidity, gas composition, light and mechanical/physical stress. In particular, packaging plays a decisive role in the improvement of fruit shelf life and new packaging materials are expected to be developed. Most of these will be derived from renewable resources (5). The application of edible coatings to freshly harvested products offers a less expensive alternative with potentially equally beneficial outcomes. The use of coatings creates a modified atmosphere surrounding the commodity similar to that achieved by controlled or modified atmospheric storage conditions. The modified atmosphere created by edible coatings can protect the food from the moment it is applied, through transportation to its final retail destination, and in the home of the consumer (6, 7, 8). Coatings made of polysaccharides have a no oily aspect, a low caloric content and can be used to increase the shelf life of fruits, vegetables, shellfish or meat products avoiding the dehydration, the oxidative rancidity and the darkening of the surface. Their application in agriculture became popular due to their permeability to CO_2 or O_2, their more convenient color, the effect of reducing weight loss, extends shelf life and can prevents microbial spoilage of the fruits (9, 10, 11). However, the effectiveness of edible coatings for fruits preservation depends primarily on the control of the wettability of the coating followed by the permeability properties and mechanical resistance. Currently the international trends demand the introduction of alternative sources of seed gums (12) and it is therefore important to search for alternative renewable sources for e.g. the production of edible and biodegradable films and coating materials. In particular, Latin American sources of galactomannans are not well known, in spite of the rich biodiversity of the local flora and of the favourable climate for their production (13).

Materials and Methods

The **seeds** of *A. pavonina* (AP) and *C. pulcherima* (CP) were collected in Campus do Pici, Federal University of Ceará-Fortaleza (Ce-Brazil) during January 2006. The seeds of *G. triacanthos* (GT) and *S.*

japonica (SJ) were collected in Botanic Garden in Porto, Portugal, during April of 2006. The **materials** used to prepare the edible coating solutions were: galactomannans, glycerol (87 %, Panreac, Spain) and distilled water. Bromonaphthalene (Merck, Germany), formamide (Merck, Germany) and ultra pure water were used to determine fruits surface properties. Acerola, pitanga, seriguela, cajá and mango were purchased from a local supermarket (Fortaleza, Ce-Brazil). All fruits were kept at 8-10 ºC until further use. The **fruits** were selected for their uniformity, size, color and absence of damage and fungal infection. Before testing, the fruits were left at room temperature (20 ºC) and their surface was cleaned with distilled water. Thin portions of the outer surface (skin) of the fruits were cut with a knife and adhered to a glass plate.

To obtain the **galactomannan** an aqueous extraction was performed. In this process, the seeds were removed from the pods, cleaned and placed in a blender, where they were mechanical broken. Following the operation the endosperm was manually separated from the germ and the hull, suspended in ethanol (purity 99.8 %, Riedel-de Haën, Germany) at 70 ºC during 15 minutes. The ethanol was decanted and distilled water was added in a proportion of 1:5 (endosperm: water), the suspension was left to rest for approximately 24 hours. Then water, in a proportion of 1:10, (suspension: water) was added and mixed in a blender for 5 min. The endosperm mixed in the blender was filtered through a nylon net followed by a centrifugation step at 3 800 g (Sigma 4K, B. Braun, Germany) during 20 minutes at 20 ºC. The precipitation of the galactomannan was achieved by adding the supernatant to ethanol (purity 99.8 %, Riedel-de Haën, Germany) at a ratio of 1:2. The ethanol was decanted and the precipitated galactomannan was lyophilized and kept in a dry place until further use.

The **intrinsic viscosity** measurements were performed in aqueous solutions prepared by stirring the mixture of galactomannans and glycerol were total solubilization was achieved. An Ubbelohde capillary viscometer of 75 mL was used. Intrinsic viscosity determinations were made in solutions of different concentrations of galactomannans and glycerol.

The **coating solutions** were prepared dissolving the galactomannans in distilled water followed by the addition of the glycerol. Each solution was stirred during 2 hours and left to stabilize during 10 minutes at room temperature.

The **critical surface tension** of the fruits as determined according to Zisman (14). In systems having a surface tension lower than 100 mN/m (low-energy surfaces), the contact angle formed by a drop of liquid on a solid surface will be a linear function of the surface tension of the liquid. The Zisman method is applicable only for low energy surfaces; therefore it is necessary to determine the surface energy of the fruits. The estimation of the critical surface tension was performed by extrapolation from Zisman plots (14). Owens and Wendt (15), Rabel (16) and Kaelble (17) demonstrated that both the tensions of liquid and solid can be separated in to polar and dispersive interactions. The liquids used to determine the surfaces properties from the fruits have: the surface tension, the dispersive and the polar component were, respectively, 72.10, 19.90 and 52.20 mN/m to water, 44.40, 44.40 and 0.00 mN/m to bromonaphtalene and 56.90, 23.50 and 33.40 mN/m to formamide (18).

When a solid is contacted by a liquid in the presence of vapour, the liquid will adhere well on the solid surface if the total free energy required for the creation of the new interface decreases. The physical significance of this energy change is the work needed to separate the solid and liquid from the solid/liquid interface, being the equilibrium the **spreading coefficient** (*Ws*). Contact angle and liquid-vapor surface tension were measured in a face contact angle meter (OCA 20, Dataphysics, Germany). The **surface tension** of the coating solution was measured by the pendant drop method using the Laplace-Young approximation (19). The samples of the coatings were taken with a 500 μL syringe (Hamilton, Switzerland), with a needle of 0.75 mm of diameter. The contact angle at the fruit surfaces was measured by the sessile drop method (20), in which a droplet of the tested liquid was placed on a horizontal surface and observed with a face contact angle meter.

The **antifungic test** was performed with four strains of fungi: *Penicillium commune*, *Penicillium crustosum*, *Penicillium roqueforti* and *Botrytis cinerea*. These standard strains were obtained from the Micoteca da Universidade do Minho (MUM), in Braga, Portugal. The stock culture was maintained at 4 ºC. In vitro antifungal activity was determined using Malt extract Agar (MEA). The fungi were cultured on MEA and its conidial suspensions were prepared by flooding a Petri dish containing a 1 to 2 week-old sporulating culture with a solution of sterile distilled water containing agar 0.2 % (w/w) and Tween 80 0.05 % (w/w). MEA was prepared and galactomannan was used to partially replace agar in a proportion of 50 % (w/w) of galactomannan and 50 % (w/w) of agar. The following procedure of autoclaving and plating was followed as before. The prepared plates containing galactomannan were inoculated with 20 µL of spore's suspension. The diameter of fungal growth was measured and used as a measure of growth inhibition during six days; the experiments were performed in triplicate. Plates containing natamicine were used as the negative control.

The **sub-chronic toxicity** has the objective of providing information of the potential risk to the health of repeated dosage of galactomannans in a limited period of time. The experiments were conduced in rats and cares were taken such as adaptation to the experimental atmosphere, and food regime. The animals were randomly distributed into three groups of five animals per sex per group and received untreated control diet mixed with control diet at dose levels 1000 mg/kg/day and 500 mg/kg/day for a period of at least three months. A control group (five individuals per sex) received untreated standard laboratory diet (control). The animals were heavy, weekly. The parameters: corporal weight, glycemic levels and total cholesterol were certain and appraised.

For the determination of the glycemic levels, the catalyzed reaction of Hexoquinase was used. The samples were picked in the anticoagulant presence. The kit of LABTEST (Centerlab, Brazil) was used and the readings made at 340 nm in a spectrophotometer. For the determination of the total cholesterol the reactions of the enzymes esterase, oxidase and peroxidase were used. The samples were collected in the presence of an anticoagulant. The kit of LABTEST (Centerlab, Brazil) was used and the readings were made in a spectrophotometer at 500 nm.

Results and Discussion

Rheological Properties

The knowledge of rheological properties, such as intrinsic viscosity, of galactomannans and their mixtures with glycerol, is important to understand the possibilities of application and the behaviour of novel galactomannan solutions as coatings. Table 1 shows the values of the intrinsic viscosity for the studied mixtures. The intrinsic viscosity increases with the decreasing degree of substitution of the backbone chain: *A. pavonina* < *G. triacanthos* < *C. pulcherrima* < *S. japonica*, which is in accordance with Cui (21). The glycerol is a small stiff molecule which will interfere with the mixture of polysaccharide and water decreasing the interactions between the polysaccharide chains (22). The synergistic interactions between the galactomannan and plasticizante decrease the intrinsic viscosity of the solutions, in the case of GT the synergistic increase the intrinsic viscosity.

Table 1 – Values from intrinsic viscosity of the galactomannans of four species of plants, with and without glycerol

Species	A. pavonina	C. pulcherrima	G. triacanthos	S. japonica
Galactomannan (no glycerol)	6.47 ± 0.45	7.22 ± 0.52	6.62 ± 0.29	9.13 ± 0.17
Galactomannan - 1 X Glycerol	3.70 ± 0.79	4.33 ± 0.67	7.31 ± 0.26	8.81 ± 0.11
Galactomannan - 4 X Glycerol	2.77 ± 0.89	3.90 ± 0.50	7.73 ± 0.20	1.88 ± 0.42

[1]Measured at the temperature of 29.06 ± 0.14 ºC (n = 5)

Surface tension and critical surface tension of fruits skins

Table 2 displays the values of the surface tension of the fruits and its polar and dispersive components. All the fruits present a higher dispersive component, which shows the ability of the fruit surface to participate in non-polar interactions. This was also demonstrated by Ribeiro et al. (8) in strawberry, where the dispersive component was higher than the polar component. Critical surface tension (Table 2) was obtained for each fruit, and varies between 9.39 and 23.92 mN/m. In all cases it is possible to conclude that the studied fruits have low energy surfaces (below 100 mN/m) meaning that the Zisman method is applicable. The obtained values are close to the critical surface tension of the apple (18.70 mN/m) and of the orange (20.00 mN/m) presented by Choi et al. (23), exception observed to acerola and pitanga that present a lower value. Also, the values of critical superficial tension must be lower than the values of superficial tension of the solid (24), which holds true for all the fruits used in this study.

Table 2 – Surface tension, polar component, dispersive component and critical surface tension of the tested fruits

Fruits	Surface tension (mN/m)	Polar component (mN/m)	Dispersive component (mN/m)	Critical surface tension (mN/m)
Acerola	27.94 ± 0.03	4.35 ± 0.01	23.59 ± 0.02	9.389 ± 0.001
Cajá	30.15 ± 0.02	2.29 ± 0.01	27.86 ± 0.01	23.923 ± 0.001
Mango	29.04 ± 0.02	1.47 ± 0.01	27.57 ± 0.01	22.678 ± 0.002
Pitanga	26.95 ± 0.02	3.07 ± 0.01	23.88 ± 0.01	13.419 ± 0.001
Seriguela	31.48 ± 0.05	4.59 ± 0.03	26.89 ± 0.02	19.622 ± 0.002

[1]Measured at the temperature of 21.27 ± 0.08 ºC (n = 20)

Wettability

Wettability determinations were performed with different galactomannan concentrations for varying plasticizer concentrations. The wettability was studied by determining the values of the spreading coefficient (Ws). The values of the spreading coefficient from the galactomannans of AP, CP, GT and SJ when applied on each fruit were analysed and are presented below. To GT and SJ were tested only in mango. For each fruit, the best (higher) value of Ws for the respective galactomannan was determined (Tukey test, p < 0.05). The best values are filled in gray. The results show that the values of Ws are quite dependent on both the source and concentration of galactomannan, and the fruit tested. In table 3, the values of Ws obtained using the galactomannan of AP are displayed.

Table 3 – Spreading coefficient (Ws), obtained for solutions of AP galactomannan and glycerol on the analysed fruits

Gal. (w/v)	Glycerol (v/v)	Acerola	Cajá	Mango	Pitanga	Seriguela
0.5	1.0	-29.92 ± 2.10 [a]	-36.50 ± 3.05 [a]	-30.97 ± 2.17 [ade]	-28.17 ± 7.27 [a]	-29.15 ± 2.78 [a]
0.5	1.5	-36.35 ± 3.95 [b]	-35.32 ± 3.74 [a]	-31.40 ± 2.97 [ade]	-31.71 ± 6.11 [a]	-23.72 ± 2.01 [b]
0.5	2.0	-42.38 ± 3.58 [c]	-27.84 ± 2.89 [b]	-37.91 ± 3.80 [b]	-39.13 ± 7.15 [bcd]	-28.95 ± 3.74 [a]
1.0	1.0	-42.11 ± 3.03 [c]	-30.80 ± 2.96 [b]	-34.37 ± 2.43 [abc]	-38.53 ± 3.91 [bcd]	-31.11 ± 2.79 [ad]
1.0	1.5	-46.71 ± 2.91 [d]	-36.96 ± 4.37 [a]	-30.93 ± 3.62 [ade]	-38.18 ± 3.78 [bcd]	-37.46 ± 2.65 [c]
1.0	2.0	-47.09 ± 4.54 [d]	-32.00 ± 3.44 [c]	-35.39 ± 1.75 [bc]	-44.22 ± 6.98 [c]	-36.86 ± 3.15 [c]
1.5	1.0	-41.57 ± 5.04 [c]	-32.85 ± 2.67 [c]	-29.18 ± 3.57 [e]	-26.45 ± 4.58 [a]	-32.35 ± 2.96 [d]

[1]Measured at the temperature of 29.06 ± 0.14 ºC (n = 5)

1.5	1.5	-41.06± 3.04 [c]	-31.24 ± 2.91 [bc]	-33.71 ± 2.95 [acd]	-32.75 ± 3.27 [ca]	-32.54 ± 3.70 [d]
1.5	2.0	-42.68 ± 1.41 [c]	-31.54 ± 3.21 [bc]	-30.38 ± 2.39 [ade]	-35.15 ± 2.68 [cd]	-37.90 ± 2.26 [c]

[a-e] Means (n = 10) in same column with different superscript are significantly different ($p < 0.05$).

When the galactomannan of CP was used, the values of Ws (table 4) present statistically significant differences for each fruit. In this case, a single solution that has the lower value of Ws was found. In all cases, with the exception of mango (the best Ws value was obtained with 1.5 % of galactomannan) the best value of Ws was obtained with values of 0.5 % of galactomannan.

Table 4 – Spreading coefficient (Ws), obtained for solutions of CP galactomannan and glycerol in the analysed fruits

Gal. (w/v)	Glycerol (v/v)	Acerola	Cajá	Mango	Pitanga	Seriguela
0.5	1.0	-42.68 ± 6,50 [a]	-27.69 ± 3.73 [a]	-51.29 ± 4.14 [a]	-40.76 ± 4,61 [a]	-40.57 ± 3.10 [a]
0.5	1.5	-39.83 ± 5,95 [a]	-31.85 ± 2.37 [b]	-65.48 ± 4.57 [b]	-35.54 ± 5,39 [b]	-36.33 ± 3.39 [b]
0.5	2.0	-32.59 ± 4,65 [b]	-38.86 ± 5.14 [ce]	-49.73 ± 6.09 [a]	-39.70 ± 3,71 [a]	-40.66 ± 2.82 [a]
1.0	1.0	-44.70 ± 4,42 [a]	-45.34 ± 4.46 [d]	-68.82 ± 6.38 [c]	-47.91 ± 6,25 [c]	-45.27 ± 3.17 [c]
1.0	1.5	-43.47 ± 3,37 [a]	-45.08 ± 3.54 [d]	-77.83 ± 5.87 [d]	-50.01 ± 4,73 [c]	-51.16 ± 3.82 [d]
1.0	2.0	-41.36 ± 3,32 [a]	-47.48 ± 4.31 [d]	-66.24 ± 7.72 [ec]	-57.02 ± 2,86 [d]	-49.87 ± 3.51 [d]
1.5	1.0	-42.38 ± 6,32 [a]	-40.06 ± 6.04 [ce]	-64.36 ± 7.84 [ec]	-41.88 ± 4,30 [a]	-41.44 ± 4.72 [a]
1.5	1.5	-40.60 ± 3,77 [a]	-37.55 ± 2.59 [c]	-62.81 ± 4.26 [e]	-43.01 ± 5,46 [a]	-42.29 ± 3.37 [a]
1.5	2.0	-58.65 ± 5,65 [c]	-43.58 ± 3.72 [ced]	-45.20 ± 4.49 [f]	-58.83 ± 5,31 [d]	-47.81 ± 3.59 [d]

[a-e] Means (n = 10) in same column with different superscript are significantly different ($p < 0.05$).

The wettability of galactomannans solutions from GT and SJ were tested on mango surface. The values of Ws (table 5) to GT solution present the best value to 1.0 % of galactomannan and 2.0 % of glycerol presenting statistically significant differences for the other solutions. In SJ case, the solution of 1.0 % of galactomannan and 1.5 % of glycerol has the lower value of Ws (statistically significant difference from other solutions). The SJ solutions of 1.5 % were not tested, the high viscosity of the solutions do not allow the application of the wettability procedure.

Table 5 – Spreading coefficient (Ws), obtained for solutions of GT and SJ galactomannan and glycerol in mango.

Gal. (w/v)	Glycerol (v/v)	*G. triacanthos*	*S. japonica*
0.5	1.0	-64.79 ± 2.72 [abc]	-56.45 ± 6.28 [a]
0.5	1.5	-66.89 ± 5.14 [ab]	-65.49 ± 5.21 [b]
0.5	2.0	-64.15 ± 2.46 [abc]	-57.97 ± 8.65 [ab]
1.0	1.0	-60.79 ± 5.13 [cd]	-61.23 ± 4.69 [ab]
1.0	1.5	-68.46 ± 1.75 [b]	-41.66 ± 4.72 [c]
1.0	2.0	-52.24 ± 4,26 [e]	-50.36 ± 6.14 [d]
1.5	1.0	-61.38 ± 3.54 [acd]	-
1.5	1.5	-57.98 ± 5.35 [d]	-
1.5	2.0	-64.34 ± 1.00 [abc]	-

[a-e] Means (n = 10) in same column with different superscript are significantly different ($p < 0.05$).

Antifungic tests

Some of the galactomannan present inhibitory effects in fungal growth. In the negative control no fungal growth happens. The galactomannan of GT presents the most favourable results. With *P. commune* the best results happen with GT. Exist statistically difference between the results for plates with galactomannan from GT and the positive control. In the other and, do not exist statistically difference with the negative control. Also to CP exist statistically difference with positive control. To *P. crustosum* the best results also happens to *G. triacanthos* and *C. pulcherrima,* existing statistically difference between there results and the positive control. To *P. roqueforti,* only GT present good results, presenting statistically difference with the positive control. In the case of *B. cinerea* GT galactomannan have the best results with statistically difference with positive control and without statistically difference with negative control, to all days of test.

Table 6 – Mean zone inhibition for the five types of plates to the four fungi's used.

	Mean zone inhibition (cm)									
	Positive Control		Negative control		*A. pavonina*		*G. triacanthos*		*C. pulcherrima*	
Time (days)	3	6	3	6	3	6	3	6	3	6
P. commune	1.9 [a]	4.0 [b]	0.5 [c]	0.5 [c]	1.9 [a]	3.5 [d]	1.0 [c]	2.3 [e]	1.5 [f]	3.1 [d]
P. crustosum	2.3 [ab]	4.4 [c]	0.5 [d]	0.5 [d]	2.2 [ae]	4.1 [c]	1.5 [f]	2.6 [b]	1.8 [ef]	3.0 [g]
P. roqueforti	2.7 [a]	6.1 [b]	0.5 [c]	0.5 [c]	2.9 [a]	6.4 [b]	1.5 [c]	4.2 [d]	2.4 [a]	5.2 [e]
B. cinerea	2.0 [a]	5.4 [b]	0.5 [c]	0.5 [c]	2.6 [ad]	10.0 [e]	0.5 [c]	1.1 [c]	2.4 [d]	4.5 [b]

[a-e] Means (n = 10) in same column with different superscript are significantly different ($p < 0.05$).

Sub-chronic toxicity

The sub-chronic was tested to the galactomannan from CP. Figure 1 presents the mean body weight of males during the three months of treatment. Comparing the means of the three groups by the Tukey test it is possible to conclude that there is no statistically difference between the three groups. No mortality occurred during this study in the case of the males.

In the group of females (Figure 1 (b)) treated with 500 mg/kg//day of CP, two have died, one at day 80 and the other at day 89. Tukey test demonstrates that there was no statistically difference between the three groups. The results show that the inclusion of galactomannan in the rats' diet does not influence their body weight.
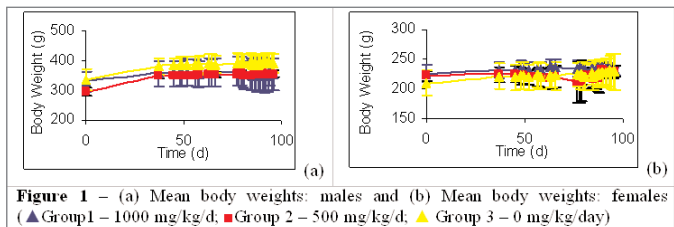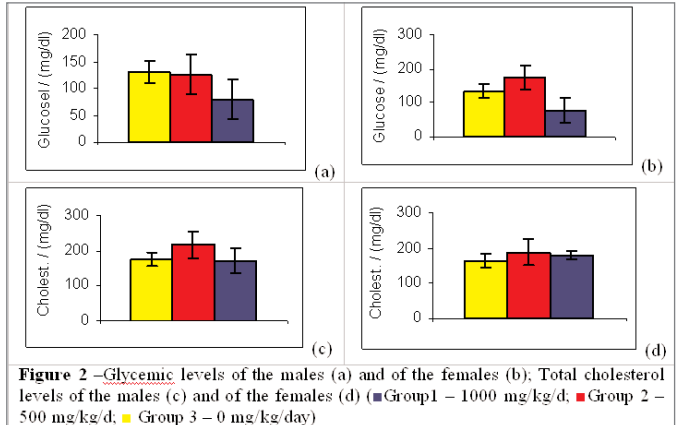

Figure 1 – (a) Mean body weights: males and (b) Mean body weights: females (▲ Group1 – 1000 mg/kg/d; ■ Group 2 – 500 mg/kg/d; ▲ Group 3 – 0 mg/kg/day)

Glycemic and cholesterol levels

The glycemic and cholesterol levels were measured with the galactomannan from CP. Figure 2(a) shows that the values of glucose for both groups having a daily amount of galactomannan of 500 and 1000 mg/Kg decrease.

In the case of the females (Fig. 2(b)), those eating a daily amount of galactomannan of 500 mg/kg show an increase of the glycemic levels comparing with the control group. In the group fed with 1000 mg/kg//day of galactomannan the value decrease. In cholesterol levels there was an increase (Fig. 2(c)) in the

groups to which the galactomannan has been fed, being the group fed with 500 mg/kg//day the one which shows a higher level of total cholesterol. Figure 2(d) show that also the females display an increase in the total cholesterol levels. In both cases (males and females) the level of total cholesterol is higher for the group that was feed with 500 mg/kg/day of galactomannan.

Figure 2 –Glycemic levels of the males (a) and of the females (b); Total cholesterol levels of the males (c) and of the females (d) (■Group1 – 1000 mg/kg/d; ■Group 2 – 500 mg/kg/d; ■ Group 3 – 0 mg/kg/day)

Application of the coating (CP) in acerola and mango fruit

After the optimization by th *Ws* of the solution of galactomannan from *C. pulcherrima*, the solution with 0.5 % galactomannan and 2.0 % of glycerol was applied on acerola. The fruit weight was measured during two days after the application of the solution of galactomannan and glycerol (respectively) when the fruit was stored at the temperature of 25.5ºC, and during six days under the storage temperature of 8.1 ºC. At a temperature of 25.5 ºC and after 2 days the mass of acerola without coating was decay 3.40 g and with coating 2.56 g. The same experiment was repeated under the temperature of 8.1 ºC in order to study the effects temperature. Also in the case that the temperature is 8.1 ºC the fruits with the coating have less lost of the weight that the fruit without coating (control). With those results we can say that at this temperature the coating go to influence the decrease of the weight from acerola. The same solution of CP was applied in mango fruits, in this case two techniques were tested; in the first the fruits were merged in CP solution and in the second case the fruits were painted. The fruit weight was measured sixteen days after the application during the storage at 25 ºC. The control and the merged fruits do not have statistically difference, on the other hand the painted show a decrease of weight loss. The visual appearance of the mango presents a big difference, with the fruits which were treated with coatings showing a much better appearance.

Conclusions

The first conclusion drawn from this study is that galactomannans can be extracted from seeds in a fast and cheap way and that they can be applied as coatings. The intrinsic viscosity of solutions from different galactomannans and glycerol were determined using a glass capillary viscometer, of Ubbelohde type. The values to the four galactomannans: *Adenanthera pavonina, Caesalpinia pulcherrima*, *Gleditsia triacanthos and Sophora japonica,* were respectively: 6.47 cm^3/g, 7.22 cm^3/g, 6.62 cm^3/g, 9.13 cm^3/g. The capacity of the seed galactomannans to decrease the fungal growth has been evaluated and there was an observable decrease in growth, especially in the presence of the galactomannan from seeds of *G. triacanthos*. The subchronic toxicity analyse from the *C. pulcherrima* galactomannan in rats, measuring the body weights, glycemic levels and total cholesterol levels, have demonstrate that this galactomannan don't have toxicity in rats. The fruit surface was characterized, being the surface tension and the critical surface measured for acerola, cajá, mango, pitanga and seriguela. The surface properties of the galactomannans were analysed in different fruits, and the mixture galactomannan/glycerol for the coating of a given fruit was optimized.

The best values of wettability were obtained for the coating compositions under study, depending on the origin of the galactomannan also on the fruit. The best coatings for the fruits were those corresponding to best values of the spreading coefficients (*Ws*). The study of weight variation of acerola during time for two different storage temperatures was performed using the best coating (0.5 % galactomannan and 2.0 % of glycerol) from *C. pulcherrima* as coatings. There was a decrease of weight loss using coatings for both temperatures tested. The study of weight variation of mango (1.5 % galactomannan (CP) and 2.0 % of glycerol) showed that the weight loss observed for different application methods of coatings does not make a significant difference, but the visual appearance of the mango presents a big difference, with the fruits which were treated with coatings showing a much better appearance. The obtained results confirmed the suitability from galactomannan based coatings to be applied in tropical fruits; further studies have to be made to study the permeability and mechanical properties from these coatings as the differences in respiration from coated and uncoated fruits.

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