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Pharmacotechnical Characterization Of Pectins Obtained From Different Varieties Of Mango From Côte d'Ivoire For Pharmaceutical Application

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Abstract

Context: Mango is one of the most common tropical fruits in the world. Its peel contains pectin, which has antioxidant and gelling properties. The objective of this work was to highlight the interest of using mango pectin in the development of pharmaceutical forms. *Methodology :* Different varieties of mangoes, available in Côte d'Ivoire, were used in this study. The extraction of the pectin from their dried and ground peel powder was carried out in an acid medium by the microwave technique. The degrees of esterification of the pectins were determined after titrimetric determination. The pH and the flow properties of the gels obtained from these pectins were evaluated respectively using a pH meter and the Malvern rotational rheometer. *Results :* The pectins obtained had a brown colour, an aromatic odor and a crystalline appearance. They had variable degree of esterification and were Low Methoxy Pectin. The gels obtained had variable pH and viscosities depending on the variety of mango, with a shear-thinning character. *Conclusion and prospect :* Mango varieties had similar characteristics but different rheological properties. Textural and stability studies will have to be carried out.

***Keywords** : Mangifera indica; LMP pectin; gels; flow properties*

Introduction

Pectin makes up around 30% of the primary cell walls of plants (Scheller et al., 2007). However, only a few plants are treated as sources of commercial pectins, depending on the yield, time and cost of extraction procedures, the desired properties of the extracted pectins and the availability of raw materials (Hui et al., 2006). Pectic substances extracted from fresh tissue or from the dry matter of fruit and vegetables vary from 0.1% to 30%, depending on the source and extraction methods. Different degrees of esterification of pectins can be obtained. The main sources are citrus fruit (6-26%), soya hulls (18-28%), mango (9-29%) and grapes (13-32%) (F. Munarin et al., 2012).. To date, pectin has mainly been produced from fruit and vegetable waste (Jayani et al., 2005; F. Munarin et al., 2012). Various extraction procedures are described, with particular emphasis on the conditions that make them more or less suitable for extracting pectins for biomedical applications. Egg carton gelation allows pectin to form a solid hydrogel in an aqueous solution. Several pectin formulations (hydrogels, films, micro- and nanoparticles) have been used to date for various proteins and active substances by varying the type and concentrations of the polymer. Some work has already been published on pectin and its applications in controlled release (Assifaoui et al., 2011; Coimbra et al., 2011; Jantrawut et al., 2013; Fabiola Munarin et al., 2012; Munarin et al., 2011, 2010, 2010; Nguyen et al., 2014).
Mango pectin was chosen not only as part of a process to recycle mango peel waste, since mangoes are Côte d'Ivoire's third largest fruit crop (Agroforestry, 2023), but also to reduce the harmful effects of this waste on the environment and health. Mango peel is also an excellent source of pectin, with both highly and weakly methylated pectins (Ajila et al., 2010). Four mango cultivars from Côte d'Ivoire (Amélie, Kent, Mangot Noukourouni and Zill varieties) were selected on the basis of their fibrous character and availability throughout the country. Different pectins were obtained from these four mango varieties and then characterised in order to formulate mango pectin gels, as the type of pectin determines the structure of the gel. Hydrogels of these pectins were prepared and characterised. The pH and rheological properties were determined.

Material and methods

The mango varieties :Amélie, Kent, Mangot Noukourouni, Zill came from the north of Côte d'Ivoire. Citric acid, sodium citrate dihydrate, NaOH, absolute ethanol and HCl, all of analytical grade, were obtained from Sigma Chemical Co (St. Louis, MO, USA). Distilled water was used throughout the study.

Harvesting mangoes and identifying varieties

The mangoes were obtained from mango exporting producers in May 2021 in Korhogo (northern Côte d'Ivoire) and identified by a botanist. They were identified at the Centre National de Floristique (CNF) in Abidjan (Côte d'Ivoire), in accordance with herbarium Nº UCJ000983-UCJ000986.

Mango processing

The mangoes were washed in soapy water and the skin removed. The mango skins were cleaned and then dried in an oven at 40°C for 48 hours. The dried mango skins were finely ground using a Grindomix GM 300 knife mill (Retsch®) and sieved using a 50 µm mesh sieve. The resulting mango peel powder was stored in jars in the refrigerator before extraction.

Extraction of mango pectin (PM)

Twenty grams of dried mango powder were mixed with 600 ml of 1N HCl solution diluted to pH 1.5. The mixture was microwaved at 500W for 20 min. The filtrates were centrifuged at 4800 rpm for 20 min to remove the remaining coarse particles. The extracted pectin was then precipitated with 95% ethanol and dried for 48 h in an oven at 40 ± 1°C, with a humidity of 35 ± 2% (Chaiwait et al., 2015). The powder was then ground and sieved using a 50 µm mesh sieve. The powder was packaged in plastic tubes and stored in a refrigerator at 4 ± 2° C before being characterised. The extraction yield (%) was calculated using the following equation: Extraction yield (%) = (Mo/M) x 100, Mo is the mass of dried pectin obtained and M is the mass of dried mango peel powder.

Determination of the degree of esterification (DE) of mango pectins

50 mg of mango pectin was dissolved in distilled water (100 mL). After adding five drops of phenolphthalein, the solution was titrated with 0.05 M NaOH until it turned pale pink; the volume of 0.05 M NaOH obtained is taken as the initial volume (V1). 10 ml of 0.5 M NaOH is added and the mixture is stirred and left to stand for 15 minutes at 30°C. The mixture was then made up with 10 ml of 0.5 M HCl and the solution stirred until colourless. A further five drops of phenolphthalein were added, and the sample was titrated with 0.5 M NaOH until a persistent pale pink (Freitas de Oliveira et al., 2016). This titration end point was recorded as the final volume (V2). ED (%) = (Final volume V2/ (Initial volume V1 + Final volume V2) x 100.

Preparation of mango pectin gels

Mango pectin gels were prepared using the modified method of Vithanage et al. (2010), 2% LMP pectins were dissolved in citrate buffer (sodium citrate dihydrate + citric acid) and heated to 65 °C with constant stirring at 800 rpm for 10 min. A solution of CaCl2 (0.2 % w/w) was then added and vigorously stirred in the solution. The gels obtained were stored in the refrigerator at 4 ± 2 °C for 24 hours before being analysed.

Measuring the pH of mango pectin gels

The pH of mango pectin gels from the four varieties was measured using a pH meter HI 2211 (Hanna Instruments, Neo-Tech SA). The pH measurements were carried out on the mango pectin gels 1 hour after cooling and removal of air bubbles at 20° C before being stored in the refrigerator.

Rheological characterisation of gels

Rheological properties were determined with the Kinesus rotary rheometer (Malvern Instruments, Massy, France), using a planar geometry with a gap of 1 mm. The instrument is fitted with a lid to cover the exposed surface of the samples to prevent drying. The planar geometry and the choice of a suitable gap of 1 mm avoided possible fracturing of the gel around the truncation. The rheological properties of the gels were determined using the smallest strain analysis of the viscoelastic properties in the linear viscoelastic region (LVR) (Doucet et al., 2001). To determine the correct parameters, the LVR was obtained using an oscillatory strain sweep at a frequency of 1 Hz under a stress range of 0.01 to 10 Pa. The tests were repeated three times using approximately 10 grams of gel each time and the mean value of the repetitions was used to calculate the rheological parameters. The values obtained were analysed using rSpace data processing software (Malvern Instruments, Massy, France).

Determination of flow properties and thixotropy as a function of shear

The evolution of the shear stress as a function of the shear rate, which varied from 0.01 to 50 s^{-1} , was used to determine the rheogram or flow curve. The dynamic shear viscosity of the gels was evaluated as a function of shear rate, which was increased from 0.01 to 50 s^{-1} over 30 s. Then, the stable shear rate of 50 s^{-1} was maintained for 30 s, followed by a downward ramp back to 0 s^{-1} in 30 s. Determination of the flow properties was carried out at 25 ± 1° C for the four gels while using the Herschel-Bulkley model for rheofluidifying fluids with a critical stress (Gilbert. 2012):

$\tau = K \gamma^n + \tau_0$, τ = shear stress (Pa); γ = shear rate (s^{-1}); n = flow index; K = consistency index ($Pa.s^n$), τ_0 = critical stress (Pa).

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Measuring the viscoelasticity of gels and oscillation test

Frequency sweeps were performed between 0.01 and 100 Hz at low strain (1%) and carried out at 25 ± 1° C. The frequency sweep profile of the gels was obtained by measuring G' and G″. The elastic modulus (G') is a measure of the energy stored and recovered per sinusoidal strain cycle. It is a measure of the elastic nature of the samples. The viscous modulus (G″) is a measure of the energy lost per sinusoidal deformation cycle. It is an indication of the viscous nature of the samples. The elastic modulus G' as a function of frequency can provide information about the characteristics of the gel (Doucet et al., 2001). The frequency dependence of the elastic modulus G' was fitted using equation (Arogundade et al., 2012) $G' = K(\omega)^n$.

Measuring the influence of temperature on the viscoelasticity of gels

The moduli G and G″ were recorded as a function of temperature using a frequency of 1 Hz and a strain of 1% to be in the linear viscoelastic region. The experiments were performed by heating the samples from 5 to 90° C with a final temperature return to 25° C with a heating rate of 5° C/min.

Statistical analysis

Each experiment was performed in triplicate and results were presented as mean ± standard deviation.

Results

Pharmacotechnical characterisation of mango pectins (PM)

The extraction yields (EY) and degrees of esterification (DE) of mango pectins (MP) are listed in Table 1. The RE and DE ranged from 12-24% and 20-31% respectively, confirming that the pectins obtained were Low Methoxy Pectin (LMP). The water content of the pectins was low at around 7%.

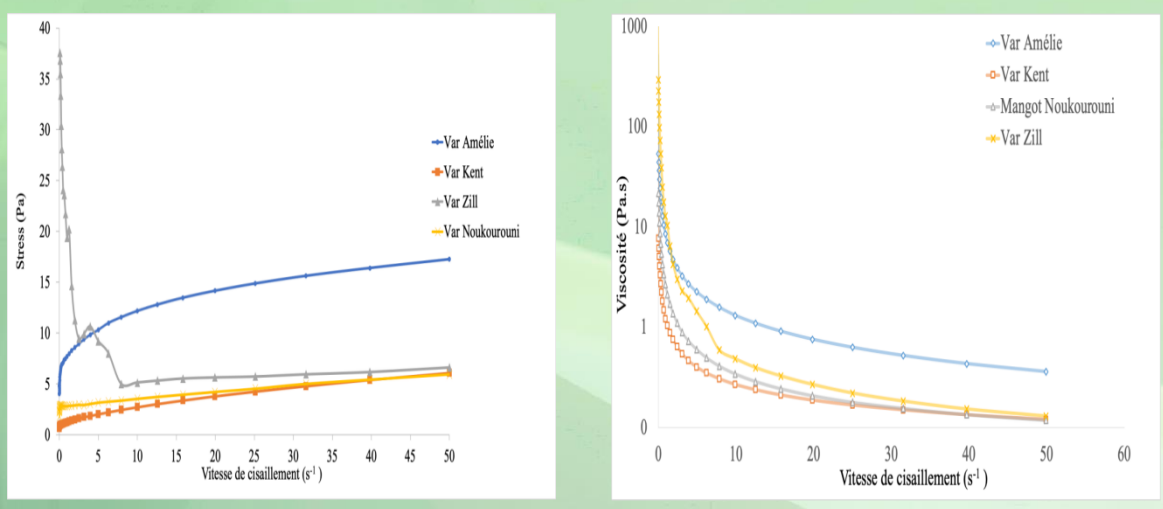
Preparation of mango pectin gels

Gels were obtained from LMP pectin solutions by adding a calcium chloride solution because the polyuronate chains of LMP could be bound by calcium to produce aggregates by the so-called "egg-box" structure, although the mechanism differs slightly from the "egg-box" model described for alginates (Grant et al., 1973). These aggregates are the precursors of gel formation reducing the solubility of pectin. Gelation occurs via the creation of junction zones by the galacturonate groups of pectin with specific sequences of galacturonic acid monomers arranged in parallel. The interaction between divalent cations and carboxylate groups in LMPs involves intermolecular bonding by chelation of the cations, leading to the formation of macromolecular aggregates. In addition, the properties of the gels obtained by the interaction between pectin and calcium are also influenced by the ED. When the ED decreases, an increase in the viscosity and firmness of the gel is observed in the presence of calcium ions (Lootens et al., 2003). The pH values of these gels are shown in Table 2. These pH values ranged from 4.56 to 4.78 (Table 2).

Rheological characterisation of gels

Determination of the flow properties of four mango pectin gels at 25 ± 1◦C.

Flow properties were determined at 25 ± 1° C in order to select a gel in which to incorporate the microparticles for further work. The mango pectin gels exhibited low critical stresses τ_0 between 0.8 and 2.5 Pa (Table 3)



(a) (b)

Figure 1. Changes in shear stress (a) and viscosity (b) of mango pectin gels as a function of shear rate at 25 ± 1◦C.

The influence of temperature on the viscosity of Amélie mango pectin gel (GA) was demonstrated at a temperature of 37 ± 1° C. Temperature and frequency influences on gel viscoelasticity (GA) was determined. The sample was equilibrated at different temperatures before the measurements were taken.

Determination of the influence of temperature on the viscosity of mango pectin gel of the Amélie variety

Mango pectin gel of the Amélie variety (GA) at a temperature of 37°C has a lower consistency index of 0.4 Pa.s (Figure 2) than the gel at a temperature of 25°C (6.5 Pa.s) (Table 3).

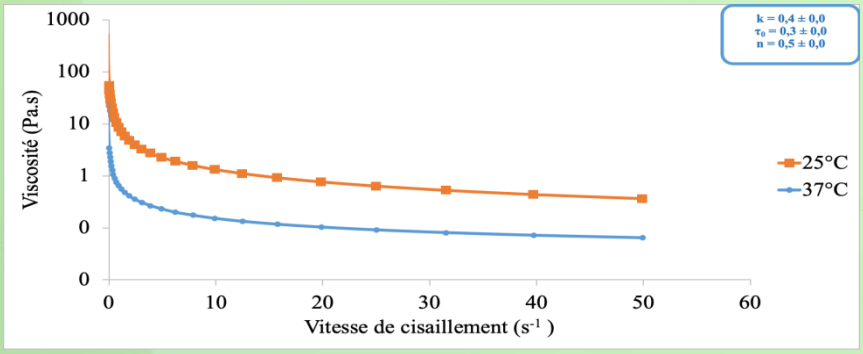


Figure 2. Viscosity of Amélie mango pectin gel as a function of shear rate at 25 ± 1 °C and 37 ± 1 °C.

Determination of the viscoelastic properties of Amélie mango pectin gel and oscillation test

The elastic modulus G' and viscous modulus G" were determined by varying the frequency between 0.01 and 100 Hz at 25 ± 1°C (Figure 3).

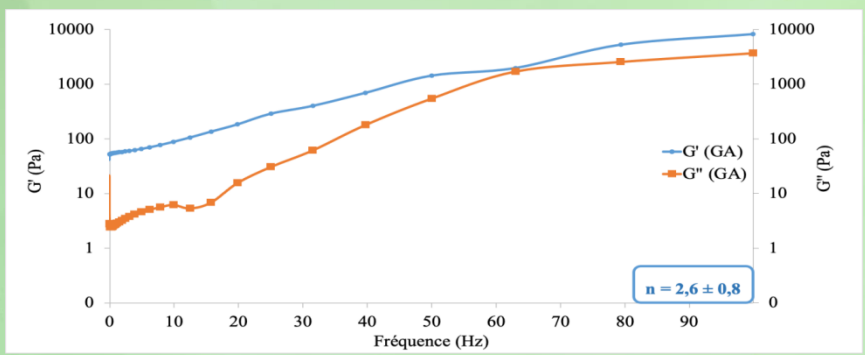


Figure 3. Frequency variation of elastic (G') and viscous (G") moduli of Amélie mango pectin gel at 25 ± 1 °C.

Determination of the influence of temperature on the viscoelasticity of mango pectin gel Amélie

Figure 4 shows the behaviour of Amélie mango pectin gel (GA) subjected to a range of heating (5-90°C). Over the entire temperature range, the GA gel showed high thermal stability, as the elastic modulus was greater than the viscous modulus (Figure 4).

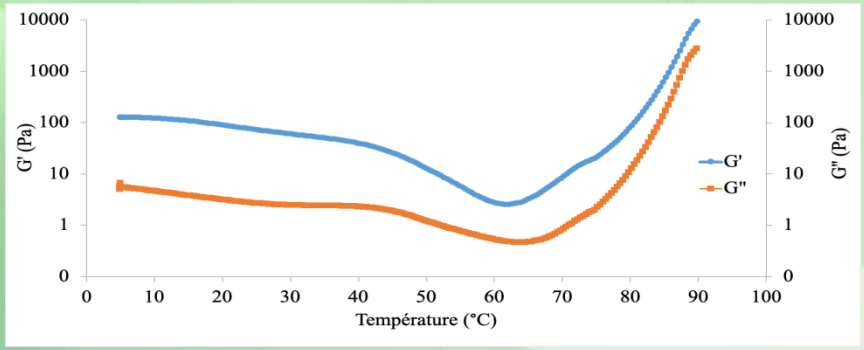


Figure 4. Changes in elastic (G') and viscous (G") moduli of Amélie mango pectin gel as a function of temperature.

Table 1. Pharmacotechnical characterisation of mango pectins (MP)

Varieties of mango	Extraction yields (%)	Degree of esterification (%)	Water content (%)
Amélie	24 ± 0,9	27 ± 0,4	7,1 ± 0,2
Kent	17 ± 0,7	20 ± 0,3	7,4 ± 0,6
Mangot noukourouni	22 ± 0,7	31 ± 0,4	7,3 ± 0,4
Zill	19 ± 1,1	31 ± 0,3	7,4 ± 0,3

Values are expressed as mean ± standard deviation, n = 3

Table 2. pH of mango pectin gels (PM)

Type of gel by mango variety	Amélie	Kent	Mangot noukourouni	Zill
pH	4,67 ± 0,37	4,65 ± 0,05	4,56 ± 0,04	4,78 ± 0,43

Values are expressed as mean ± standard deviation, n = 3

Table 3. Rheological parameters of mango pectin gels, experimental data based on the Herschel-Bulkley rheological model at 25 ± 1°C

Type of gel by mango variety	K (Consistency index in Pa.s)	N (Flow index)	τ_0 (Critical stress in Pa)
Amélie	6,5 ± 2,6	0,2 ± 0,0	2,2 ± 1,4
Kent	0,5 ± 0,0	0,6 ± 0,0	0,8 ± 0,0
Mangot noukourouni	0,2 ± 0,0	0,8 ± 0,0	2,5 ± 0,1
Zill	20,1 ± 8,1	0,0 ± 0,0	0,0 ± 0,0

Values are expressed as mean ± standard deviation, n = 3

Discussion

The yield and type of mango pectin depend on the cultivar and extraction conditions. The pectin obtained was weakly methylated and can form gels in the presence of divalent cations, making it an ideal carrier for the delivery of bioactive agents (Colodel et al., 2019; Huynh, 2017). According to pH, similar values were found for LMP pectin hydrogels (Moreira et al., 2014). These pH values were close to the pH of the scalp (5.5) because an alkaline pH can increase the negative electrical charge on the surface of hair fibres and, consequently, increase friction between fibres (Gavazzoni Dias et al., 2014).

Rheogical characterization showed that Zill gel appears to have the highest viscosity at low shear rates (Figure 1). The dynamic viscosities decreased as a function of shear rate (0.01-50 s^{-1}), demonstrating their rheofluidising behaviour (Figure 1).

This rheofluidifying character was also highlighted by the values of the flow index (n), which ranged from 0.0 to 0.8 and were less than 1 (Table 3). This decrease in viscosity can be interpreted by the separation and gradual alignment of entangled macromolecules during flow.

This rheofluidifying behaviour was observed in studies on the rheological analysis of gels obtained from eight mango cultivars from different regions (Deng et al., 2020). It was also similar to that of apple pomace pectin gels (Zhang et al., 2013), sugar beet pulp (Chen et al., 2015) and grapefruit peel (Wang et al., 2016). Mango pectin gel of the Amélie variety (GA) was selected for further work because it had a consistency of 6.5 Pa.s (neither soft nor hard gel), which made it possible to include microparticles, and a pronounced rheofluidifying character (n = 0.2) (Table 3). At 37°C, thermofluidification of mango pectin gel of the Amélie variety was observed. This result is due to disentanglement of the macromolecules under the influence of the increase in temperature.

At all frequencies, the elastic modulus was greater than the viscous modulus (Figure 3). The gel behaved essentially like an elastic fluid, confirming the firmness of the Amélie variety mango pectin gel (Han et al., 2017).This result was due to inter-chain entanglement of the molecular pectin (Piermaria et al., 2008). This result is similar to the results obtained on agarose gels as strong polysaccharide gels, due to the elastic modulus in these polysaccharides being greater than the viscous modulus at all frequencies (Ross-Murphy, 1995). However, at a frequency of 63 Hz, the elastic and viscous moduli are equal (Figure 3). Pectin gel is therefore not viscoelastic. When G′ is plotted as a function of ω (angular frequency), the degree of dependence of G′ can be determined by the parameters of the power law given by the relation $G' = K(\omega)^n$. According to this equation, K is a constant and n is the slope in a log-log plot of G′ as a function of ω. The parameter n can provide information about the structure of the gel. In covalent gels, n = 0, whereas in physical gels n > 0. Thus, (n) is related to the strength and nature of the gel (Colodel et al., 2019). Such an analysis makes it possible to identify covalently cross-linked materials and physical gels. Therefore, n can be used as a measure of the characteristics of the physical gel versus the covalently cross-linked gel (Gunasekaran and Ak, 2000; Zhang et al., 2017). In general, food gels exhibit slightly frequency-dependent behaviour, implying that they are physical in nature (Renard et al., 2006). The GA gel obtained was of a physical nature (because n = 2.6) with ionic bonds between the galacturonic units of pectin and calcium (Figure 3).

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Over the entire temperature range, the dominant elastic character of the GA gel observed previously was confirmed. No gel-soil transition point was observed. However, a decrease in the values of the elastic and viscous moduli when the GA gel was heated was observed from 5 to 65°C. Above 65°C, the opposite effect was noted.

Conclusion

The gels were prepared from mango pectin obtained by microwave extraction from dried mango skins of four cultivars from Côte d'Ivoire in order to select the pectin that would be suitable for further work. The extraction yields of mango pectins ranged from 12 to 24% and their water content was low (7%). The degrees of esterification of mango pectins obtained by titrimetry varied between 20 and 31%, confirming their low methylation potential (LMP), since the degrees of esterification were less than 50%. The preparation of gels from each of the different low-methyl mango pectins (LMP) was carried out in the presence of Ca^{2+} because the polyuronate chains of low-methyl pectins (LMP), together with divalent cations, lead to egg-box gelling and produce stronger gels. The gels obtained were the subject of various characterisations which led to the selection of the Amélie variety mango pectin gel (GA). The pH of the GA gel was 4.67, close to that of the scalp (5.5) and favourable for skin tolerance. Rheological characterisation of the GA gel was carried out at 25°C (ambient temperature) and 37°C (body temperature). At 25°C, the gel showed a consistency of 6.5 Pa.s, a rheofluidifying character ($n = 0.2$) that will facilitate the spreading of the gel on the hair and a low stress τ_0 that will allow the gel to flow easily. At 37°C, the influence of temperature was noticeable with a consistency of 0.4 Pa.s, which reflects a thermofluidification of the gel under the effect of the increase in temperature with a lower stress τ_0 . The increase in temperature did not alter the rheofluidifying character of the gel. Whatever the frequencies and temperatures applied, the GA gel showed a dominant elastic behaviour testifying to its firmness. It also appeared to be a physical gel with ionic bonds (because $n > 1$) depending on the frequencies.

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