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Characterization and physicochemical properties of dietary fiber concentrates as potential prebiotic ingredients for use in fish nutrition

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Abstract

Dietary fibers are formed by non-starch polysaccharides as cellulose, hemicellulose, pectins, gums, mucilages, β-glucans, among others. These constituents have prebiotic properties and are therefore not digested in the gut, reaching intact in the colon and altering the microflora of the colon. In developing, beneficial microflora produces physiological effects capable of improving the life of the host. Thus, the knowledge of the biological and functional properties of dietary fibers has led to the development of methods of obtaining these compounds for possible use in animal nutrition. Then, this study aimed to obtain dietary fiber concentrates (DFC) from different agro-industrial sources and evaluate their respective chemical composition and physicochemical properties. The DFC - mucilage, pectin, and βglucan + mannan (βG+M) were obtained from linseed, citrus pulp, and brewer's yeast (*Saccharomyces cerevisiae*), respectively, through different physicochemical processes. The chemical composition revealed that the predominant component in all DFC were dietary fiber and the insoluble fraction. The DFC that obtained most extraction yield was βG+M (19.81% ± 8.54), followed by pectin (14.54% ± 2.72), and mucilage (7.18% ± 1.54). The mucilage and pectin composition have greater monosaccharide diversity since the βG+M consisted primarily of mannose (74.5%) and glucose (24.3%). The pectin showed numerically lower hydration capacity than the other DFC. For the oil binding ability, all DFC had similar values. In this study, the DFC presented nutritional and technological characteristics that indicate potential application of the agro-industrial sources as a prebiotic for fish supplementation.

Keywords: β-glucan+mannan. Fish feeds. Linseed. Mucilage. Pectin.

Caracterização e propriedades físico químicas de concentrados de fibras alimentares como potenciais ingredientes prebióticos para uso na nutrição de peixes

Resumo

As fibras alimentares são formadas por polissacarídeos não amiláceos como celulose, hemicelulose, pectinas, gomas, mucilagens, β-glicanos, entre outros. Estes constituintes têm propriedades prebióticas e, portanto, não são digeridos no intestino, atingindo intactos e alterando a microflora do cólon. Quando desenvolvida, a microflora benéfica produz efeitos fisiológicos capazes de melhorar a vida do hospedeiro. Desta forma, o conhecimento das propriedades biológicas e funcionais das fibras alimentares levou ao desenvolvimento de métodos de obtenção desses compostos para possível uso em nutrição animal. Logo, o presente estudo teve como objetivo a obtenção de Concentrados de

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Fibras Alimentares (CFAs) a partir de diferentes fontes agroindustrial e avaliar suas respectivas composição química e propriedades físico-químicas. O CFAs (mucilagem, pectina e βglicana + manana (βG+M)) foram obtidos a partir da linhaça, polpa cítrica e levedura de cervejaria (*Saccharomyces cerevisiae*), respectivamente, através de diferentes processos físico-químicos. A composição química revelou que o componente predominante em todos os CFAs foram fibra alimentar e a fração insolúvel. O CFA que obteve maior rendimento de extração foi βG+M (19.81% ± 8.54), seguido pela pectina (14.54% ± 2.72), e mucilagem (7.18% ± 1.54). A composição da mucilagem e pectina tiveram maior diversidade de monossacarídeos, uma vez que a βG+M consistiu principalmente de manose (74.5%) e glicose (24.3%). A pectina apresentou numericamente menor capacidade de hidratação que os demais CFAs. Para a capacidade de ligação ao óleo, todos os CFAs apresentaram valores similares. Neste estudo, os CFAs apresentaram características nutricional e tecnológica que indicaram potencial de aplicação das fontes agroindustrial como um prebiótico para a suplementação de peixes.

Palavras-chave: β-glucana+manana. Alimentação de peixes. Linhaça. Mucilagem. Pectina.

Introduction

For a long time, dietary fibers represented the inert portion of food because of their low-energy content. However, the interest in dietary fibers has been increasing due to their beneficial effects on the microflora of the gastrointestinal tract (Bach Knudsen, 2001; Wenk, 2001; Montagne *et al*., 2003).

Dietary fibers are formed by non-starch polysaccharides, among which we highlight cellulose, hemicellulose, pectins, gums, mucilages, β-glucans, among others (Chen *et al*., 1988; Mudgil and Barak, 2013). These components have been receiving a great deal of attention because of their prebiotic properties, as they are not digested in the intestine and remain intact when they reach the colon and are metabolized by beneficial bacteria, which alter the colonic microflora. This leads to a healthy bacterial microflora capable of inducing important physiological effects on the health and well-being of the host (Catalani *et al*., 2003).

In the last five decades, the cultivation of aquatic organisms has increased steadily (FAO, 2014). Thus, several strategies have been adopted to ensure an increase in production and stock breeding health, including the use of antibiotics as growth promoters in feeds. However, the use of these products has been restricted due to their potential for the development of resistant bacteria, hazards to the environment, suppression of the immune system of the animals and risk of bioaccumulation in fish (Ringo *et al*., 2010). For these reasons, the use of alternative growth promoters in feeds, particularly prebiotics, is currently recommended. Moreover, adding prebiotics in fish nutrition is also important because they are substances rather than living organisms. Therefore, they are more resistant to processing as well as extrusion and pelletization (Névoa *et al*., 2013).

Knowledge of the biological and functional properties of dietary fibers has recently led to the development of methods of obtaining these compounds for use in animal nutrition. For this reason, the present study aimed to obtain dietary fiber concentrates, with prebiotic potential to apply to fish nutrition, from different agro-industrial sources and evaluate their chemical composition and physicochemical properties.

Materials and methods

The study was conducted in the Fish Farming Laboratory, Department of Animal Science of the Universidad Federal of Santa Maria (Santa Maria, RS, Brazil).

Raw material

Yeast biomass (*Saccharomyces cerevisiae*) was kindly provided by Santamate Indústria e Comércio Ltda (Santa Maria, RS, Brazil). Linseed (*Linum uistatissimum L.)* was donated by Giovelli & Cia Ltda (Guarani das Missões, RS, Brazil). The citrus pulp, composed by rind or flavedo, albedo, membranes, and seeds, was processed in our laboratory.

Obtention of dietary fiber concentrates

β-glucan+mannan was obtained from brewer's yeast *(Saccharomyces cerevisiae*) according to the methodology described by Goulart *et al*. (2017a) and Chaud *et al*. (2007), with some modifications. Initially, aqueous yeast extract was centrifuged at 3500 rpm (15 min) and washed three times with distilled water at a 1:1 ratio (w/v). After this, the extract was subjected to autolysis at 49°C for 8 h and was centrifuged at 3500 rpm (15 min). The supernatant was discarded and the precipitate was collected, which correspondent the cell wall. This fraction was submitted to an alkaline treatment with NaOH 1% (1:3 w/v), under agitation and heating (75°C/20 min). Subsequently, the sample was neutralized with HCl 2N, centrifuged at 3500 rpm (15 min), and washed three times with distilled water at a 1:1 ratio (w/v). The final precipitate was represented by the β-glucan+mannan fraction, which was dried at 40°C for 24 h.

The procedure of mucilage extraction from linseed was performed according to Goulart *et al*. (2013) in an aqueous medium (10% w/v), at 60 to 80°C with constant agitation, for 150 min. The supernatant was

removed and ethanol (final concentration of 75% v/v) was added in order to precipitate the fiber fraction. The precipitate was collected, dried in a forced-air-drying oven at 60°C/24 h, and ground in a laboratory mill.

Pectin was obtained from the citrus pulp according to the method described by Calliari (2015). Before extraction, the orange juice residue (flavedo, albedo, membranes, and seeds) was washed with water, crushed manually, dried in a forced-air-drying oven (50°C/24 h), and ground in a laboratory mill to obtain the dried citrus pulp. Then, pectin was extracted in an aqueous medium (8% w/v) at 100°C for 1 h. After cooling, the mixture was centrifuged (3500 rpm/10 min), the precipitate was discarded, and ethanol was added to the supernatant at a 1:1 ratio (v/v) to precipitate the pectin. This mixture was left at 5°C for 24 h in order for pectin to be precipitated. Finally, the precipitate was dried in a forced-air-drying oven (55°C/24-48 h) and ground in a laboratory mill.

Chemical composition analysis

Samples, linseed, mucilage, dried citrus pulp, pectin, brewer's yeast (*Saccharomyces cerevisiae),* and β-glucan+mannan, were analyzed to determine dry matter (DM) (number 930.15), ash (number 942.05), and crude protein (CP - N x 6.25) according to AOAC (1990) methods (number 954.01). Fat was measured according to Bligh and Dyer method (1959). Total dietary fiber (TDF), soluble fiber (SF), and insoluble fiber (IF) content were determined according to the enzymatic gravimetric method (number 991.43) (AOAC, 1995).

Extraction yield

The extraction yield was calculated by the ratio between the final weight product and the weight of sample submitted to extraction.

Monosaccharide composition

The determination of monosaccharide composition was carried out according to Biermann (1989). Initially, samples were hydrolyzed with trifluoroacetic acid 1 M for 5 h at 100°C. Upon completion of the hydrolysis, the excess acid was removed by evaporation. After total acid hydrolysis, the monosaccharides were solubilized in distilled water and reduced by adding, approximately, 10 mg of sodium borohydride for 16 h at 4°C (Wolfrom and Thompson, 1963b). Alditols were submitted to acetylation (Wolfrom and Thompson, 1963a). The extraction of alditol acetate was performed by the addition of chloroform and subsequent elimination of pyridine in successive treatments with 5% copper sulphate and distilled water. After solvent evaporation, the alditol acetates were subjected to gas-liquid chromatography (GLC) for determination of neutral monosaccharide composition.

The resulting alditol acetates were analyzed by GLC in a Trace GC Ultra chromatograph (Thermo Electron Corporation- EUA) equipped with a DB-225 (0.25 mm x 30 m) capillary column. The injector and flame ionization detector (FID) temperatures were 250°C and 300°C, respectively. The oven temperature was set from 100 to 215°C, at a heating rate of 40°C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min.

Uronic acids content were measured by the method of Blumenkrantz and Asboe-Hansen (1973). Galacturonic acid was used as standard solution and the absorbance was measured at 520 nm.

Physicochemical properties

The water holding capacity (WHC) and oil binding capacity (OBC) were determined according to McConnell *et al.* (1974) and Abdul-Hamid and Luan (2000), respectively. Sample (1.0 g) was weighed, loaded into graduated test tube, 20 mL of distilled water (for WHC) or oil (for OBC) was added, and the mixture was stirred until complete homogenization. Then, it was left at room temperature for 24 h. After, the mixture was centrifuged (3500 rpm, 1 h), the supernatant was removed, and the tube (sample + absorbed water or oil) was weighed. The results were expressed as the amount of water or oil retained by the sample per gram.

Results and discussion

Chemical composition

All resulting dietary fiber concentrates (DFC) showed high total dietary fiber (TDF) content (Table 1), which proved that the methodologies used for fiber concentration were suitable for the proposed aim. For mucilage obtained from linseed, Monego (2009) mentioned higher levels for TDF (85.07%) and SF (73.21%). The contrast in results can be explained by differences in the variety of the raw material employed as well as by the procedure used for obtaining the food products (Kaewmanee *et al*., 2014).

Although pectin had the lowest TDF, this DFC had the highest SF content (31.47 ± 1.75). This fraction presents an important role for the natural microflora of colon. In the intestine, soluble fibers are fermented easily and more quickly than the insoluble fibers (Puupponen-Pimiä *et al*., 2002; Catalani *et al*., 2003). Short-chain fatty acids (SCFA) are produced through fermentation (Saad, 2006) and they are responsible for various benefits to the host, such as regulation of epithelial proliferation and differentiation of the colonic mucosa and improvement of blood flow and mucus production. Additionally, SCFA are the preferred energy source for the colonocytes, decrease pH in the colon, maintain balance in the intestinal microflora, and provide beneficial effects on sodium and water absorption, lipid and glucose metabolism, pancreatic secretion, and other hormones (Catalani *et al*., 2003).

Table 1 – Chemical composition of the raw samples and the dietary fiber concentrates.

Components	Samples					
	Linseed	Mucilage	Citrus pulp	Pectin	Yeast	βG+M
TDF	53.09±10.11	61.73±3.25	47.52±2.08	32.76±1.05	11.88±2.38	64.18±0.31
IF	38.19±8.74	39.50±5.44	25.37±0.39	1.29±0.71	1.32±0.15	60.98±0.00
SF	14.90±1.37	22.23±2.19	22.16±1.69	31.47±1.75	10.56±2.23	3.2±0.31
CP	16.09±0.11	11.29±0.95	5.67±0.25	5.10±0.15	39.44±0.10	10.31±0.46
Fat	31.59±0.57	2.67±0.08	7.65±0.00	0.56±0.11	2.24±0.45	1.20±0.11
Moisture	7.76±0.23	13.25±0.21	11.51±0.01	15.39±0.15	68.76±0.79	10.55±0.00
Ash	3.41±0.09	6.65±0.05	3.25±0.13	3.62±0.03	7.49±0.33	7.41±0.15

Results are expressed as mean ± standard deviation (SD) (n=3). TDF: total dietary fiber; IF: insoluble fiber; SF: soluble fiber; CP: crude protein; βG+M: βglucan+mannan.

For β-glucan+mannan, an increase was observed in TDF content compared to in nature yeast. Similar content (69.7%) was reported by Chaud *et al.* (2007). However, these authors reported that 60.2% of this fiber is in the soluble form, which is contrary to the findings of the present study. The results suggested that higher levels of insoluble fibers are associated with the shape of the β-glucan present in the yeast. According to Magnani and Castro-Gómez (2008), there are two fractions of β-(1-3) glucan on the cell wall of *Saccharomyces cerevisiae,* one soluble and the other insoluble. The insoluble portion represents the largest cell wall component, while the soluble portion accounts for 15 to 20%. Furthermore, Sinha *et al.* (2011) reported that mannans are highly insoluble polysaccharides in water and very dense.

In works carried out by Adorian *et al.* (2019), Goulart *et al.* (2017a) and Goulart *et al.* (2017b) positive results were observed on the growth, metabolism and immune system of jundiás (*Rhamdia quelen*) fed with diets supplemented with dietary fiber concentrates, demonstrating that they are potential candidates to exert prebiotic effect on fish nutrition, since prebiotics are known to act by stimulating the absorption of certain nutrients, altering microbial metabolism, and in addition to increasing the levels of antibodies and macrophage activity (Saad, 2006).

In relation to other nutrients, the procedures employed for fiber concentration reduced the fat content. The decrease is probably due to the treatment used for fiber solubilization, combined with the hydrophilic nature of the solvent, has not caused translocation of fat to the resulting extract. Likewise, CP content was reduced in the resulting dietary fiber fractions. For moisture and ash content, the mean values found in mucilage and pectin were similar to those found in the literature (Cui and Mazza, 1996; Kliemann *et al.*, 2009). However, Chaud *et al.* (2007) reported an ash content 40 to 50% lower than the findings found in the present study. Nevertheless, in general, all the DFC had low ash content, which is a good indication of sample purity (Kliemann *et al.*, 2009).

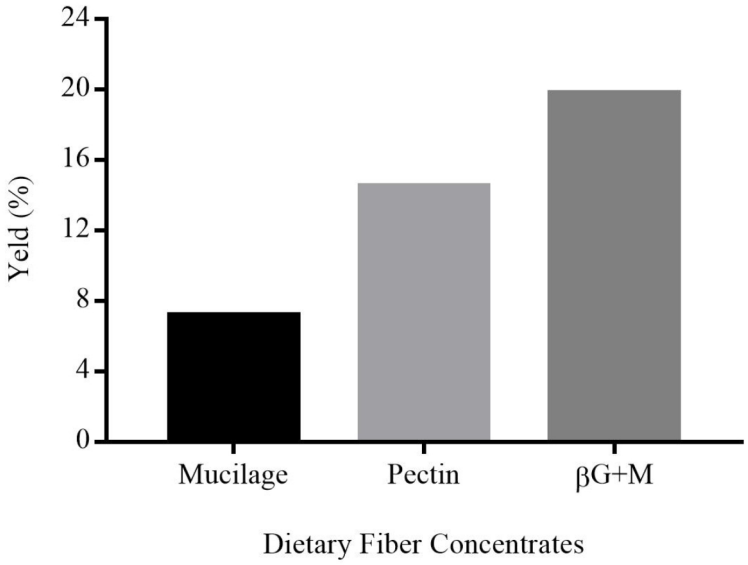
Extraction yield

The extraction yields of the different DFC obtained from agro-industrial sources are shown in Figure 1. The DFC that had the highest extraction was βG+M with a mean of 19.81% yield. In the literature, there is little data that allow a comparison of the extraction yield of this fraction. Published studies assessed the fractions in isolation, as in the work by Chaud and Sgarbieri (2006), who obtained 25.13% extraction yield for mannans and 42.92% for glucans from the cell wall of semi-purified *Saccharomyces cerevisiae*. For pectin, the extraction yield was 14.54%. On the other hand, Calliari (2015) evaluated various methodologies for pectin extraction from citrus pulp and observed higher yields for acid extraction (39.23% for citric acid and 26.70% for acetic acid). For pectin obtained from passion fruit peel, citric acid was also the best extracting agent, resulting in about 70% yield. In the same study, the extraction yield was lower for nitric and hydrochloric acids - 38 and 26%, respectively (Kliemann *et al.*, 2009). Thermal extraction of pectin was not as efficient as acid extraction, which is generally used in the food industry (Canteri *et al.*, 2012) and has higher extraction yields. However, this procedure can be considered a cost-effective and environmentally friendly method because it does not generate toxic waste in the environment and has low cost. The industries has faced an aggravation to the environment, the residues generated in the production, therefore it is urgent the adoption of actions that aim to reduce the generation of residues.

The extraction yield of mucilage, 7.18%, was consistent with other results found in the literature (Qian *et al.*, 2012). Fedeniuk and Biliaderis (1994) tested different methods to extract of linseed mucilage and observed a lower extraction yield (3.6%) using low-temperature water (4°C). On the other hand, when applying higher

temperatures the mucilage had higher yield (9.4%), which is similar to the findings in the present study. Likewise, Cui *et al.* (1994) observed a higher extraction yield of mucilage using high temperatures between 85-90°C, pH 6.5-7.0, and water:seed ratio equal to 13. It is suggested that the high temperatures cause the removal of a greater amount of free water, increasing the extraction yield (Kaushik *et al.* 2017). Besides the afore mentioned features, the extraction yield of mucilage depends on the culture environment and variety of this grain (Qian *et al.*, 2012).

Figure 1 – Extraction yield for mucilage, pectin, and βglucan+mannan (βG+M) obtained from agro-industrial sources.



Monosaccharide composition

The monomers that comprise the fibers are classified as pentoses - arabinose and xylose, hexoses - glucose, galactose and mannose, 6-deoxihexoses - rhamnose and fucose, and uronic, glucuronic, and galacturonic acids (Meurer and Hayashi, 2003). In addition, knowledge of the polysaccharide composition is extremely important, because the physiological impact of the fibers depends on the sugar residues and the nature of the connections between these residues (Sinha *et al.*, 2011).

In the present study, mucilage and pectin have the greatest diversity of monosaccharide (Table 2). The monosaccharide composition present in mucilage was similar to the findings in the literature (Fedeniuk and Biliaderis 1994; Oomah *et al.*, 1995; Qian *et al.*, 2012). Among the monosaccharide present, xylose, galactose and arabinose were found in a greater amount. According to Ringo *et al.* (2010), xylose oligomers (xylooligosaccharides) promote the bifidobacteria growth acting as prebiotics. Similarly, galactose molecules (galactooligosaccharides) and arabinose + xylose (arabinoxylo-oligosaccharides) have been widely used as prebiotic source for fish feeds.

Table 2 – Monosaccharide composition of dietary fiber concentrates obtained from different agro-industrial sources.

	Components (%)							
	Rha	**Fuc**	**Ara**	**Xyl**	**Man**	**Gal**	**Glc**	**UA**
Mucilage	11	3	15.5	35.4	0	17.1	6.9	11.1
Pectin	2.2	0	21.6	1.2	1	9.4	42.9	21.7
βG+M	0	0	0	0	74.5	1.2	24.3	0

Results are expressed as mean (n=3). Rha: rhamnose; Fuc: fucose; Ara: arabynose; Xyl: xylose; Man: manose; Gal: galactose; Glc: glucose; UA: uronic acid; βglucan+mannan.

Glucose, arabinose, and galactose were the main neutral monosaccharide found in pectin. For commercial pectin, Müller-Maatsch *et al.* (2016) obtained a similar composition - higher levels of glucose, galactose, and rhamnose, but in different concentrations than the findings in the present study. According to BeMiller and Huber (2010), the composition and properties of pectin vary according to their source, the process used during preparation, and subsequent treatments. Regarding the functionality of non-starch polysaccharides present in pectin, Canteri *et al.* (2012) reported that the beneficial effects of the pectin chain can be attributed to its ability

to be transformed into short chain fatty acids by the action of pectinolytic enzyme-producing bacteria (*Aerobacillus*, *Lactobacillus*, *Micrococcus,* and *Enterococcus)*. Hotchkiss *et al.* (2003) analyzed the in vitro fermentation of oligosaccharides derived from pectin extracted from Valencia oranges and concluded that these components exert bifidogenic effects and promote the increase of acetate, propionate, and butyrate after fermentation.

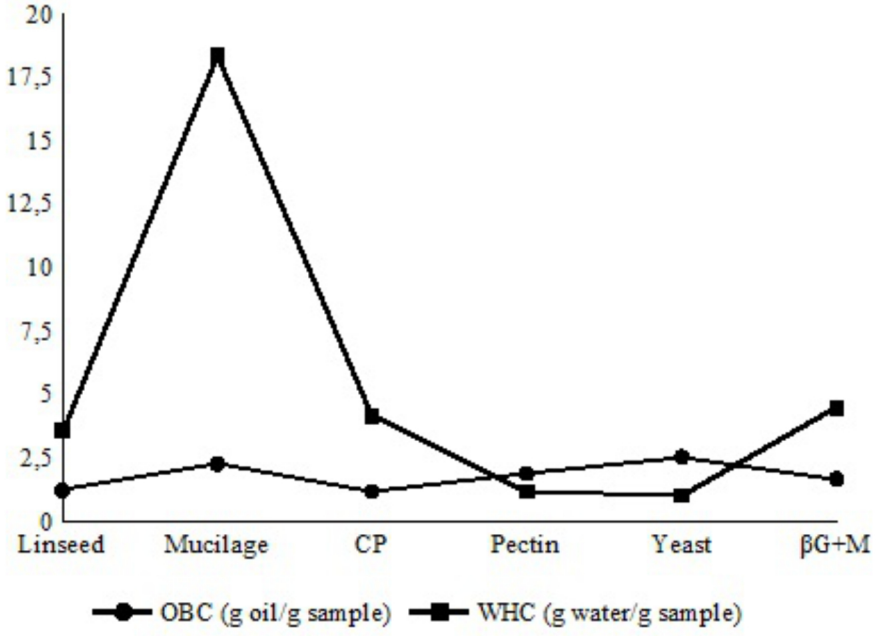
For βG+M, monosaccharides found in larger amounts were mannose (74.5%), followed by glucose (24.3%), and galactose (1.2%). According to Pinto (2012), some monosaccharides, as xylose and galactose, are associated with mannoproteins, which may be a probable explanation for the galactose content found in the βG+M fraction. In relation to the beneficial effects of the components present in βG+M, the mannose units (mannanoligosaccharide) are responsible for increasing the performance of fish and feed efficiency. Additionally, these constituents offers protection against pathogens by leveraging the local and systemic immune system and strengths gut integrity and functionality (Sinha *et al*., 2011; Torrecillas *et al*., 2014). β-glucan has shown potential prebiotic due to its immunomodulatory effect. When it is recognized by specific cellular receptors, enhances the immune response of the host (Magnani and Castro-Gómez, 2008).

Physicochemical properties

The physicochemical properties of dietary fibers have important metabolic and physiological effects on the body of animals. This is directly related to source of fiber and processing. Processing can result in important changes that must be taken into account, depending on the final destination and the properties of the product intended for commercialization (Zaragoza *et al*., 2001).

The values found for water holding capacity (WHC) and oil binding capacity (OBC) are shown in Figure 2. In this study, mucilage showed high WHC. In biological conditions, high levels of fiber intake coupled with high water holding capacity causes greater bolus volume, more satiety, increased viscosity of solutions in the gastrointestinal tract, delayed gastric emptying, among other effects (Brito *et al*., 2008; Souza *et al*., 2008), reflecting negatively on the zootechnical performance of fish. These characteristics are not desirable in fish nutrition.

Figure 2 – Physicochemical properties of the raw samples and the dietary fiber concentrates. CP: citrus pulp; βG+M: βglucan+mannan; Yeast: *Saccharomyces cerevisiae*.



Although pectin had the highest SF content, this fraction had lower WHC than raw citrus pulp. It is suggested that pectin had this behavior due to lower TDF content. Furthermore, Macagnan *et al*. (2014) observed lower WHC for orange pulp, even with higher SF levels compared with the other ingredients. This author attributed the result to the low amount of dietary fiber and the presence of free pectin in this byproduct.

The βG+M fraction had higher WHC compared with in nature yeast. This behavior may be related to the structure of β-glucan, because unlike cellulose, bindings between glucose units are variable, causing a branched structure and smaller size. These properties influence their solubility, allowing them to form viscous solutions, and acquire greater hydration capacity (Mudgil and Barak, 2013).

The OBC quantifies how much lipid a fiber is capable of absorbing. This physicochemical property is associated with the fiber's ability to bind substances in the intestine, as well as bile salts, acids, and cholesterol (Souza *et al*., 2008). There was a small increase in OBC for pectin and mucilage, and a slight reduction for βG+M (Figure 2). However, OBC was similar for all DFC obtained in the present study. Thus, it is suggested that regardless of the origin of the extracted fiber, the behavior of binding to substances in the intestine is similar.

Monosaccharide composition, the nature of binding between monosaccharides, solubility, and physicochemical properties are features that directly affect the functional properties of polysaccharides (Tavernari *et al*., 2008; Bemiller and Huber, 2010).

Conclusion

Based on the results, all dietary fiber concentrates obtained from different agro-industrial sources showed nutritional and technological properties that indicate potential application as prebiotic ingredients for fish feeds.

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Ethics Committee Approval

The study was approved by the Animal Ethics Commission of the Federal University of Santa Maria under number 23081.009051/2014-53.

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