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A Comparative Study of the Effects of Non-starch Polysaccharide Gums on Physical Properties of Single-screw Extruded Aquafeed

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Abstract

A factorial experimental design (5×3×2) was used to investigate the effects of non-starch polysaccharide binding agents on physical properties of single-screw extrusion. Extrusion cooking trials were performed with an ingredient blend for yellow perch, fortified with five non-starch polysaccharide binding agents including three plant-origin gums (guar, wheat gluten, carboxymethyl cellulose (CMC)) and two microbial-origin exopolysaccharide gums (xanthan and pullulan), with three levels of gum inclusion (3, 6, and 10%), and two levels of screw speed (100 and 150 rpm). Effects of the independent variables on extrudate characteristics were extensively analyzed and included density, expansion ratio, water absorption and solubility indices, pellet durability, and color. Increasing gum level from 3 to 10% led to a considerable increase in unit density of extrudates for xanthan, guar, wheat gluten, CMC, and pullulan by 39.6%, 21%, 11.4%, 30%, and 19.7%, respectively. The minimum (357 kg m^{-3}) and maximum (607 kg m^{-3}) bulk densities were observed for the diets produced with 6% guar at 150 rpm and 10% xanthan at 100 rpm, respectively. The mean values of expansion ratio for the diets containing exopolysaccharide gums were slightly smaller than those of the other diets. Increasing gum inclusion level increased the expansion ratio of the extrudates using xanthan, wheat gluten and pullulan but reduced the expandability of diets used guar gum; increased levels did not change the expansion ratio of extrudates containing CMC. At the highest levels of gum inclusion and screw speed, both pullulan and wheat gluten gums provided better expandability of the feed extrudate. Exopolysaccharide gums resulted in extrudates with significantly higher pellet durability and water solubility indices. Overall, the addition of 6 to 10% non-starch exopolysaccharides could improve the pellet durability of aqua feed extrudates. A future study investigating the effects of feed composition and additional extrusion processing conditions on the physical parameters of these products in aquafeeds would be appropriate.

Keywords: Aquafeed; Cohesiveness; Extrusion; Pullulan gum; Physical property

Introduction

Feed composition can vary depending upon the aquatic species' requirements and preferences. Some cultured fishes are active surface feeders such as tilapia *Oreochromis spp.* [1] and channel catfish *Ictaluris punctatus* [2], others such as yellow perch *Perca flavescens* are slightly more passive and feed throughout the water column, and yet other species such as shrimp (e.g., *Penaeus* spp.) are slow and intermittent bottom feeders [3]. Therefore, nutritional and physical properties are important in the manufacturing of aquafeed to maximize feed conversion and growth performance of the targeted species and life stage. This diversity makes the aquafeed manufacturing process more complex when compared to feed production for other food animals. The primary physical properties of aquafeed are: high water stability and pellet durability, uniformity of particle size and shape, maximum density, floatability, expansion ratio, and cohesion. Thus, the ingredients of feed should be selected based on their effective nutrient contributions and their functional properties during the feed processing [4].

To accommodate the need for production of a diverse array of feeds, extrusion technology provides the most effective and flexible processing methods for the aquafeed industry. The quality of extruded aquafeed is drastically influenced by the extruder operating parameters such as temperature, moisture content, and residence time as well as the effects of hardware structure of the extruder [5]. The interactive effects of high temperature, shearing forces, and moisture content of the blend during this process transform the feed ingredients at macroscopic and microscopic levels leading to structural changes of protein and starch. The extent of heat treatment and starch transformation affect overall cohesion of fish feed extrudates in terms of water stability and pellet durability. High water stability prevents loss of nutrients from rapid disintegration of pellets in water and increases the duration of feed availability [6]. Higher pellet durability enhances the mechanical strength of the extruded feeds to protect against the abrasive and external forces experienced during transportation. In addition to selecting proper ingredient compositions and processing parameters, one way to enhance the cohesive property of extruded feeds is by using a polysaccharide binding agent in the feed blend. In extrusion processing, starch is the most common binding agent which is responsible for most of the mechanical properties of the extrudate [7]. However, different sources of starch vary in their chemical compositions (i.e. ratio of amylose and amylopectin groups) and can result in different extrudate characteristics [8].

Several researchers reported that fish, particularly carnivorous species [9], have limited capacity to utilize dietary carbohydrates [10,11]. With the growing interest in replacing fish meal with plant-based proteins, which are typically rich in soluble and insoluble carbohydrates, there can be a risk of excess soluble indigestible carbohydrates which may interfere with nutrient digestibility, particularly lipids [12]. On the other hand, the cohesiveness and water stability of the extruded feeds are achieved due to starch gelatinization. This macromolecular transformation requires excessive heating which may impact the quality and bioavailability of the other ingredients, according to Dominy et al.

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The binding potential of several natural (i.e. casein, wheat gluten, palm starch, and sorghum starch), modified (i.e. hexa meta phosphate, alginate, and manucol), and synthesized (i.e. bentonite, urea formaldehyde, and polyvinyl alcohol) binders on water stability of pelleted shrimp feed were evaluated by Lim and Cuzon [4]. In another research, Dominy and Lim studied the effect of various commercial binders on water stability and pellet durability of shrimp feeds [13]. Chen and Jenn suggested that phospholipid supplementation can significantly improve the nutritional and physical stabilities of shrimp feed in water. Brinker studied the effect of guar gum inclusion in a plant-based trout diet and observed significant change in the fecal properties [14]. Later on, the positive effects of maltose on improving water stability and reducing nutrient leaching of pelleted fish feed were also reported by Ighwela et al. [12].

One way to reduce the carbohydrate content of aquafeeds is to consider the inclusion of non-starch polysaccharide binding agents or gums. In addition to the effect of these binding agents on nutritional quality of the feeds, their contributions and performances during the extrusion processing as well as their effects on the physical properties of the extruded aquafeed are also very critical. Non-starch polysaccharide gums can be classified into two groups of plant-origin gums such as guar gum, carboxymethylcellulose, wheat gluten, etc. and microbial-origin (exopolysaccharide) gums such as pullulan and xanthan. Nonetheless, there is scarce research on the effect of non-starch exopolysaccharide gums on the physical properties of aquafeeds. Hence, the specific objective of this study was to characterize and compare the behavior of exopolysaccharide gums and plant-origin polysaccharide gums under various single-screw extrusion processing conditions.

Materials and Methods

Feed blend preparation

Five different ingredient blends were formulated with graded levels (3, 6, and 10% db) of various non-starch polysaccharide binders including xanthan, wheat gluten, guar, carboxymethylcellulose (CMC), and pullulan, along with appropriate quantities of Menhaden fish meal, whole wheat flour, soybean oil, to mimic a practical feed formulation for yellow perch. Micronutrients (i.e., vitamin and mineral premixes) and other nutritional supplements were not included in extrusion blends since they do not have any impact on the physical properties of the feeds. The selected non-starch polysaccharide gums were of plant-origin, except for xanthan and pullulan gums which were microbial exopolysaccharides. Fish meal was purchased from Omega Protein, Houston, TX; xanthan and guar gums were obtained from Purebulk, Roseburg, OR; corn protein concentrate was supplied by Cargill, Inc., Dayton, OH; hard winter wheat flour was obtained from Bob's Red Mill Natural Foods, Milwaukie, OR; carboxymethylcellulose was obtained from USB Corporation, Cleveland, OH; pullulan was donated by Nagase America, New York, NY; soybean oil was donated by South Dakota Soybean Processors, Volga, SD; and menhaden fish meal was obtained from Omega Protein, Houston, TX.

All ingredients except gums were ground with a Fitzpatrick comminutor (Elmhurst, IL) with 1 mm screen prior to dry blending. Dry ingredients were blended for 20 min using a V-10 mixer containing an intensifier bar (Vanguard Pharmaceutical Machinery, Inc., Spring, TX). Dry blended feedstuffs were then transferred to a Hobart HL200 mixer (Troy, OH) where oils and water (20 ± 2% db) were added and blended for ~5 min. The blends stored at ambient temperature overnight for moisture balancing prior to extrusion process.

Extrusion cooking process and experimental design

Extrusion cooking of fish feeds formulated with the selected gum sources was conducted using a laboratory scale single-screw extruder (Model PL 2000 Brabender Plasti-Corder, South Hackensack, NJ) with a short barrel of 31.75 cm and L/D ratio of 20:1 consisting of three independent heating zones equipped with a compressed-air cooling system. Extrusion trials were performed with five gum sources (xanthan, wheat gluten, guar, carboxymethylcellulose and pullulan), 2 levels of screw speed (SS) (100 and 150 rpm), and 3 levels of gum inclusion (GL) (3, 6, and 10%). To acquire more distinct results for the effect of utilized non-starch polysaccharide gums– with an emphasis on exopolysaccharide gums– the barrel temperature gradient (feed zone-compression zone-metering/cooking zone) , blends' moisture content, die nozzle dimension and screw compression ratio were kept constant at 100°C-120°C-140°C, 20% db, L/D: 5.83 and screw compression ratio of 3:1, respectively. A total of 30 extrusion trials were completed and each trial was replicated only once. Approximately 1 kg of the extrudates was collected at steady state condition when the screw was rotated at a constant speed in response to the input torque (for 5 min). The screw speed, input torque and the barrel temperature ingredients were controlled by a computer control system. All the extruded diets were air dried for 48 h before physical property testing.

Extrudate physical properties

Functional or physical properties of the extruded fish feeds for treatment combinations were measured following the standard procedures used in the previous study by Fallahi et al. [15]. Unit density (UD, kg m^{-3}) was measured as the ratio of mass to volume for randomly chosen extrudates, given the assumption of cylindrical shape for the extruded diets [16]. Accordingly, volume of each extrudate was calculated from extrudate diameter and height. Bulk density (BD, kg m^{-3}) was measured with a standard bushel tester (Seedburo Equipment, Chicago, IL, U.S.A.) as the ratio of the mass of the extrudates (kg) occupying a given bulk volume to the volume of the bulk (m^3) [17]. Expansion ratio (ER) or diametral expansion of the extrudate is defined to be the ratio of the extrudate diameter to the diameter of the die nozzle (3.0 mm) [18] which was measured using a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan). Pellet durability index (PDI, %) was measured according to ASAE standard method S269.4 [19]. Using a pellet durability tester (PDI-110, Seedburo Equipment), a known amount of extruded diets (200 ± 5 g) was tumbled inside the tester for 10 min and then sieved manually with a screen (No. 6) to remove fines. Then, the PDI was calculated as the percentage of the mass (g) of the extrudates remained on the screen after tumbling. Water absorption index [WAI, (-)] and Water solubility index (WSI, %) were determined according to the method proposed by Jones et al. [20]. Stirred water stability (WS, min) was determined by recording the time duration that extrudates were stable when spun around. Approximately, 1g of extrudates for each diet was placed in 200 mL of distilled water and stirred with a magnet stirrer (Southeast Science, Model H4000-HS, Korea) at low speed, which simulates the movement of water of pond, until the extrudates broke or disintegrated [21]. Color scores included *L* (brightness/darkness), *a* (redness/greenness), and *b* (yellowness/blueness) were measured with a Minolta Chromameter (Model CM 2500d, Minolta, Japan).

Statistical analysis

Each physical property was measured with 3 replications except for density and expansion ratio which were measured with 10 replications. All data were analyzed using Microsoft Excel 2010 and SAS version 9.0

software (SAS Institute, Cary, NC, USA). Analysis of Variance with type I error rate of α=0.05 was used to determine if significant differences resulted among the diet treatments. If a significant difference was detected, then post host LSD tests were employed to determine where the specific differences occurred.

Results and Discussion

The effects of different non-starch exopolysaccharide and polysaccharide gums with graded levels of inclusion on the functional characteristics of extruded yellow perch feeds were investigated. Gum type had a significant effect on water absorption, water solubility and pellet durability indices (Tables 1 and 2). Gum inclusion level had a significant effect on all measured parameters, whereas, changing the levels of screw speed from 100 to 150 rpm did not significantly influence the functional properties of the extrudates (Table 3). All of the extrudates were water stable for at least 25 min. In addition to the effect of inclusion level of gums and screw speed, the compositions of the experimental diets by itself could impact the physical properties of the extrudates. The effect of diet compositions need to be considered in future studies.

Unit density (UD, kg m^{-3})

As shown in Table 3, only UD of the extrudates contained xanthan gum was changed with the increasing level of gum inclusion from 3% to 6%; elevating gum level from 3% to 10%, significantly increased unit density values of extrudates containing xanthan, guar, wheat gluten, CMC, and pullulan by 39.6%, 21%, 11.4%, 30%, and 19.7%, respectively. The highest unit density of 1214 kg m^{-3} was observed for the extrudates containing 10% xanthan which were extruded at screw speed of 100 rpm (Table 4). Treatment combination effect of 3% CMC and screw speed of 150 rpm resulted in extrudates with the lowest unit density of 821 kg m^{-3}. The interaction effect of GL×SS on unit density values of the extrudates containing wheat gluten and pullulan was significant, Pr <0.0001 (Table 5).

Typically, unit density of the extrudate which is affected by the extent of starch gelatinization, dictates the expandability of the products during extrusion processing as well as the buoyancy of the feeds. It is possible that the hydration of the non-starch polysaccharide gum limits the water availability and adversely affects the starch gelatinization. In this study, the differences among the unit density values of the extrudates indicate the different hydration capacity of the gums. However, the effect of extrusion conditions on starch crystalinity and water mobility restriction by gum cannot be neglected. Brennan et al. reported that adding guar gum to extruded breakfast cereals increased density of the extruded products. They suggested that guar gum may have interfered with the expansion process and swelling capacity of starch upon exiting the die.

Bulk density (BD, kg m^{-3})

Bulk density is the other important property of the extrudates commonly influenced by expansion phenomena occurring upon exiting the extrudates from die section. The size, volume, and pore shape irregularities inside the extrudates generated during water evaporation and extrudate structuring affect the bulk density of the extrudates [22].

	Xanthan			Guar			Wheat Gluten			CMC			Pullulan		
Blend Ingredient (g/100g), db	3%	6%	10%	3%	6%	10%	3%	6%	10%	3%	6%	10%	3%	6%	10%
Fishmeal, menhaden	41.7	41.7	41.7	41.7	41.7	41.7	41.7	41.7	41.7	41.7	41.7	41.7	41.7	41.7	41.7
Gum	3.0	6.0	10.0	3.0	6.0	10.0	3.0	6.0	10.0	3.0	6.0	10.0	3.0	6.0	10.0
Corn protein concentrate	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8	8.8
Whole wheat flour	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Cellufil	25.4	22.4	18.4	25.4	22.4	18.4	25.4	22.4	18.4	25.4	22.4	18.4	25.4	22.4	18.4
Soybean oil	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
Totals	100.0	100.1	100.1	100.0	100.1	100.1	100.0	100.1	100.1	100.0	100.1	100.1	100.0	100.1	100.1
Proximate analysis of blend															
Protein	37.5	37.5	37.5	37.6	37.7	37.9	39.8	42.1	45.2	37.5	37.5	37.5	37.5	37.5	37.5
Lipid	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Ash	11.3	11.6	12.0	11.0	11.1	11.1	11.0	11.0	11.1	11.0	11.0	11.0	11.0	11.0	11.0
NFE	42.2	41.9	41.6	42.4	42.2	42.0	40.2	37.9	34.7	42.6	42.6	42.6	42.5	42.5	42.5

Table 1: Ingredient components in feed blends containing 3, 6, and 10% gum, and mean proximate composition of each.

Variable	**Property**								
	UD (kg/m^3)	**BD (kg/m^3)**	**ER (-)**	**WAI (-)**	**WSI (%)**	**PDI (%)**	**L (-)**	**a (-)**	**b (-)**
Gum (-)									
Xanthan	1011^a	461^a	0.99^a	5.12^a	10.96^b	71.63^b	48.5^a	5.9^a	23.9^a
	(180)	(95.0)	(0.07)	(1.80)	(4.90)	(16.80)	(2.85)	(1.44)	(2.99)
Guar	994^a	424^a	1.02^a	6.02^a	11.30^b	58.09^c	50.3^a	5.6^a	23.6^a
	(111)	(82.0)	(0.12)	(1.70)	(5.40)	(13.50)	(5.64)	(0.96)	(1.97)
Wheat Gluten	1054^a	442^a	1.03^a	3.18^b	11.12^b	70.65^b	48.6^a	5.9^a	23.4^a
	(96)	(105.0)	(0.13)	(0.55)	(6.30)	(15.40)	(4.50)	(1.22)	(1.81)
CMC	1035^a	443^a	1.04^a	4.86^a	9.96^b	71.53^b	49.5^a	5.8^a	24.1^a
	(208)	(76.0)	(0.23)	(1.78)	(2.88)	(8.70)	(7.27)	(1.33)	(3.07)
Pullulan	1017^a	446^a	0.99^a	2.92^b	17.31^a	88.11^a	48.0^a	5.2^a	20.3^b
	(106)	(52.0)	(0.13)	(0.70)	(7.78)	(6.31)	(4.46)	(0.40)	(3.67)

where, UD: Unit density; BD: Bulk density; ER: Expansion ratio; WAI: Water absorption index; WSI: Water solubility index; PDI: Durability index; L: Lightness; a: redness; b: yellowness; Parentheses indicate ±1 standard deviation; means followed by similar letters for a given dependent variable are not significantly different at $P < 0.05$ among treatments.

Table 2: The main effect of source of gum on extrudates' physical properties.

Parameter	UD(kg/m^3)	BD(kg/m^3)	ER (-)	WAI (-)	WSI (%)	PDI (%)	L (-)	a (-)	b (-)
Xanthan									
Gum Level (%)									
3	860.0^{c}	386.0^{c}	0.96^{b}	3.78^{b}	7.19^{b}	55.5^{c}	47.1^{b}	5.10^{b}	22.4^{b}
	(40.0)	(4.0)	(0.03)	(0.23)	(1.17)	(1.64)	(1.63)	(0.09)	(0.30)
6	980.0^{b}	404.3^{b}	0.95^{b}	4.06^{b}	8.30^{b}	65.6^{b}	46.5^{b}	4.92^{b}	21.7^{b}
	(170.0)	(4.0)	(0.06)	(0.34)	(1.17)	(2.20)	(0.55)	(0.13)	(0.41)
10	1200.0^{a}	591.0^{a}	1.07^{a}	7.52^{a}	17.4^{a}	93.7^{a}	51.9^{a}	7.91^{a}	27.5^{a}
	(110.0)	(18.0)	(0.03)	(0.33)	(1.78)	(4.00)	(1.90)	(0.52)	(2.50)
SS(rpm)									
100	1036.0^{a}	468.0^{a}	0.97^{b}	5.00^{a}	11.6^{a}	70.8^{a}	48.0^{a}	5.77^{a}	23.1^{a}
	(208.0)	(104.0)	(0.07)	(1.88)	(5.51)	(15.60)	(2.17)	(1.27)	(1.94)
150	986.2^{a}	453.3^{a}	1.02^{a}	5.23^{a}	10.4^{a}	72.5^{a}	49.0^{a}	6.18^{a}	24.7^{a}
	(161.0)	(91.0)	(0.05)	(1.89)	(4.78)	(18.90)	(3.47)	(1.64)	(3.73)
Guar									
Gum Level(%)									
3	920.0^{b}	375.4^{b}	1.12^{a}	4.23^{c}	7.56^{b}	53.6^{b}	46.8^{b}	5.03^{b}	22.4^{b}
	(39.0)	(7.0)	(0.05)	(0.28)	(1.03)	(0.86)	(1.85)	(0.13)	(0.13)
6	948.3^{b}	362.0^{b}	0.88^{c}	5.78^{b}	8.73^{b}	45.3^{c}	49.8^{ab}	4.90^{b}	22.6^{b}
	(73.0)	(5.0)	(0.08)	(0.64)	(2.41)	(0.82)	(1.64)	(0.12)	(0.57)
10	1113.3^{a}	534.4^{a}	1.07^{b}	8.05^{a}	17.6^{a}	75.3^{a}	54.2^{a}	6.88^{a}	25.8^{a}
	(43.0)	(29.0)	(0.09)	(0.22)	(4.30)	(6.44)	(8.31)	(0.38)	(2.05)
SS(rpm)									
100	972.3^{a}	419.2^{a}	1.03^{a}	6.00^{a}	12.2^{a}	56.5^{a}	52.2^{a}	5.58^{a}	23.9^{a}
	(131.0)	(67.0)	(0.08)	(1.86)	(7.07)	(10.40)	(7.30)	(0.94)	(2.64)
150	1015.4^{a}	428.6^{a}	1.02^{a}	6.04^{a}	10.4^{a}	59.7^{a}	48.3^{a}	5.61^{a}	23.2^{a}
	(87.0)	(98.0)	(0.14)	(1.68)	(3.48)	(16.50)	(2.38)	(1.04)	(0.96)

Table 3: Main effects of gum inclusion level, and screw speed (SS) on extrudates physical properties blocked according to gum source; Parentheses indicate ±1 standard deviation; means followed by similar letters for a given dependent variable are not significantly different at $P < 0.05$ among treatments.

From commercial perspective, bulk density of biomaterials plays crucial role in designing the storage facilities and packaging for economic warehousing and transportation. Extrudates having higher BD requires less space which facilitates transportation of more products in smaller containers and reduces the total shipping cost [23].

As shown in Table 3, increasing gum incorporation level from 3% to 6%, led to a 4.7% increase and a 7.2% decrease in BD values of the extrudates contained xanthan and CMC, respectively; while, it did not affect the BD values of the extrudates produced using the other binders (i.e. guar, wheat gluten, and pullulan). However, further increasing of gum level to 10%, significantly increased BD of all extruded feeds (α=0.05). This is to say that increasing the non-starch polysaccharide inclusion resulted in product wall thickening. The minimum and maximum BD values of 357 kg m^{-3} and 607 kg m^{-3} were related to the diets produced with 6% guar at 150 rpm and 10% xanthan at 100 rpm, respectively. No significant differences were detected among treatments with 10% gum inclusion level and screw speed of 150 rpm for BD values of extrudates contained xanthan, guar, and CMC as the binding agents (Table 4). Whereas, pullulan inclusion in yellow perch feed at all levels significantly changed BD of the extrudates. These differences reflect the specific rheological behavior of the gums, their chemical compositions and their interactions with amylose and amylopectin during the extrusion cooking [24]. As mentioned previously, all these variables can be influenced by extrusion processing parameters such as temperature and pressure. In this study, extruder temperature and blend moisture contents were held constant. Also, the overall statistical analysis did not show any considerable change in BD of the products due to the individual effect of type of non-starch polysaccharide gum used in this study and varying level of screw speed. Table 4 shows that the combination effect of GL and SS at their highest level resulted in significantly denser extrudates, regardless of type of binder. Analyses across all collected data confirmed that there were significant differences for BD values of extrudates due to interaction effects of independent variables, except for the BD value of extrudates containing pullulan (Table 5).

Expansion ratio (ER)

As presented in Table 3, the mean values of the expansion ratio for the diets containing exopolysaccharide gums (i.e. xanthan and pullulan) were smaller than those of the other diets. It was anticipated that the magnitude of expansion would be reduced by gum inclusion due to competing effect of gums for free water of the expanding matrix and lowering its expansion capacities.

When xanthan was used as the binding agent, the expansion ratio ranged from 0.94 to 1.08. It ranged from 0.85 to 1.15, 0.89 to 1.22, 0.94 to 1.21, and 0.89 to 1.19 for the diets used guar, wheat gluten, CMC, and pullulan gums as binding agent, respectively (Table 4). As illustrated in Figure 1, at screw speed of 150 rpm, expansion ratio values for three experimental diets containing 10% pullulan, 10% wheat gluten gums and 3% CMC were not significantly different at α=0.05. The highest (1.22) and the lowest (0.85) ER values were observed for the treatment combinations of 28 (10% wheat gluten-150 rpm screw speed) and 22 (6% guar-150 rpm screw speed), respectively. These differences in expandability of the extrudates can be explained by the different contribution of non-starch polysaccharides in bubble formation upon exiting the die as observed by Brennan et al. [24]. They also reported that the inclusion of guar gum had no significant effect on the expansion ratio of the extruded breakfast cereals [25]. Wheat gluten may interact with amylose and amylopectin of the starch component of the blend during heating, which can lead to increased dough paste

Parameter	UD(kg/m^3)	BD(kg/m^3)	ER (-)	WAI (-)	WSI (%)	PDI (%)	L (-)	a (-)	b (-)
Wheat gluten									
Gum Level (%)									
3	1013.0^b	375.2^b	0.99^b	2.64^c	7.44^b	61.3^b	46.5^b	5.09^b	22.6^b
	(33.0)	(3.0)	(0.12)	(0.07)	(0.40)	(2.06)	(1.37)	(0.22)	(0.60)
6	1021.0^b	365.3^b	0.93^b	3.02^b	6.35^c	58.9^c	44.8^c	5.07^b	21.8^c
	(123.0)	(5.0)	(0.03)	(0.01)	(0.23)	(1.67)	(1.05)	(0.05)	(0.56)
10	1127.4^a	585.0^a	1.16^a	3.88^a	19.6^a	91.7^a	54.6^a	7.56^a	25.8^a
	(69.0)	(16.0)	(0.08)	(0.05)	(0.25)	(1.80)	(0.93)	(0.23)	(0.38)
SS(rpm)									
100	1038.0^a	435.0^a	1.05^a	3.20^a	11.3^a	70.2^a	49.3^a	5.96^a	23.3^a
	(131.0)	(101.0)	(0.08)	(0.58)	(6.50)	(15.00)	(4.50)	(1.21)	(1.83)
150	1070.0^a	449.0^a	1.00^a	3.18^a	10.9^a	71.1^a	48.0^a	5.85^a	23.5^a
	(79.0)	(113.0)	(0.16)	(0.57)	(6.64)	(16.70)	(4.70)	(1.28)	(1.89)
CMC									
Gum Level(%)									
3	913.0^b	406.8^b	1.08^a	3.54^b	8.04^b	76.1^a	43.3^c	4.97^b	21.9^b
	(269.0)	(17.0)	(0.39)	(0.29)	(0.76)	(4.93)	(2.38)	(0.41)	(0.88)
6	1006.0^b	377.7^c	0.99^a	3.82^b	8.11^b	60.8^b	46.1^b	4.93^b	22.3^b
	(131.0)	(14.0)	(0.05)	(0.50)	(0.84)	(3.89)	(0.75)	(0.15)	(0.46)
10	1186.3^a	543.6^a	1.05^a	7.21^a	13.7^a	77.6^a	59.1^a	7.6^a	28.1^a
	(84.0)	(23.0)	(0.05)	(0.48)	(0.78)	(3.02)	(1.21)	(0.48)	(1.43)
SS(rpm)									
100	1070.3^a	445.8^a	1.00^a	5.20^a	9.87^a	72.8^a	48.7^a	5.8.0^a	24.2^a
	(111.0)	(60.0)	(0.06)	(1.85)	(3.41)	(7.03)	(7.87)	(1.40)	(3.64)
150	999.8^a	439.6^a	1.08^a	4.51^a	10.1^a	70.3^a	50.3^a	5.86^a	24.0^a
	(27.0)	(93.0)	(0.31)	(1.82)	(2.56)	(10.40)	(7.00)	(1.34)	(2.61)
Pullulan									
Gum Level(%)									
3	937.3^b	402.2^b	0.88^c	2.61^b	11.4^b	80.8^c	45.3^b	5.29^a	22.7^a
	(43.0)	(5.0)	(0.04)	(0.04)	(1.35)	(0.73)	(1.33)	(0.12)	(0.60)
6	992.5^b	438.3^b	0.96^b	2.36^b	12.8^b	87.8^b	44.9^b	5.35^a	22.5^a
	(89.0)	(57.0)	(0.08)	(0.04)	(0.64)	(0.83)	(1.91)	(0.32)	(1.65)
10	1122.0^a	498.7^a	1.15^a	3.80^a	27.7^a	95.7^a	53.8^a	4.85^b	15.7^b
	(78.0)	(8.0)	(0.06)	(0.45)	(0.80)	(0.87)	(1.38)	(0.50)	(2.27)
SS(rpm)									
100	1023.4^a	436.0^a	0.96^a	3.03^a	17.1^a	88.1^a	48.0^a	5.36^a	21.4^a
	(125.0)	(46.0)	(0.10)	(0.87)	(7.81)	(6.68)	(4.40)	(0.29)	(3.14)
150	1011.0^a	457.2^a	1.03^a	2.82^a	17.6^a	88.1^a	48.0^a	4.97^b	19.2^a
	(85.0)	(57.0)	(0.14)	(0.54)	(8.49)	(6.32)	(4.79)	(0.42)	(3.98)

Table 4: Main effects of gum inclusion level, and screw speed (SS) on extrudates physical properties blocked according to gum source; Parentheses indicate ±1 standard deviation; means followed by similar letters for a given dependent variable are not significantly different at *P*<0.05 among treatments.

viscosity during extrusion affecting expansion phenomena. Typically, unit density of the extruded feeds is inversely related to the extent of expansion ratio of the products during extrusion processing; however, our results did not support their findings. As the unit density increased, expansion ratio of the extrudates was also increased for all types of gums evaluated. Although speculation, it would be reasonable to assume that the difference in expansion behavior of non-polysaccharides used in this study could be due to their interactions with the starch component of the mixture which caused different plasticization behavior and thus differed their physicochemical transformations during extrusion.

Several studies have been conducted to investigate the effect of different binders on water stability and performance of pelleted aquafeed [26,27]. To our knowledge, there is no published literature on the effect of extrusion processing and non-starch polysaccharide gum inclusion on expandability of aquafeeds. Solomon et al. studied the effect of different starch-based binding agents (wheat grain starch, corn

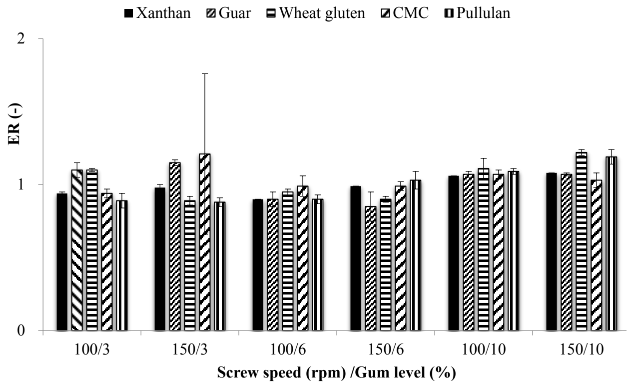

Figure 1: Effect of gum, gum level, and screw speed on expansion ratio (± standard deviation; bars with different letters were significantly different).

grain starch, millet grain starch, guinea corn grain starch and cassava tuber starch) with yeast *Saccharomyces cerevisae* on the floatation property of fish feed [28].

Water absorption (WAI)

Typically, water absorption of extrudates can be attributed to structural changes in polymeric (i.e. protein and starch) and/or non-polymeric (i.e. lipid) macromolecular components of the feed blend. Increasing the gum levels from 3% to 6% did not change the WAI values for xanthan, CMC, and pullulan extrudates but did result in increased WAI values by 36.6% and 14.4% for guar and wheat gluten extrudates, respectively (Table 3). Moreover, with an increase in gum content from 6% to 10%, a substantial increase in WAI values by of 85.2%, 39.3%, 28.5%, 88.7%, and 61% was observed for xanthan, guar, wheat gluten, CMC, and pullulan gum extrudates, respectively. The WAI values ranged from 3.77 to 7.64 for xanthan gum extrudates, from 4.0 to 8.10 for guar gum extrudates, from 2.64 to 3.88 for wheat gluten extrudates, from 3.29 to 7.86 for CMC extrudates, and from 2.33 to 4.11 for pullulan gum extrudates (Table 4). The highest WAI value (8.10) was obtained for extruded diets containing guar gum followed by xanthan gum and the lowest WAI value (2.33) was for extruded diets containing pullulan gum. The different chemical structure of the gums is likely the main reason of their varying water holding capacities after extrusion cooking. Solubilization of the oligosaccharide components of these polysaccharides during the extrusion could affect the water adsorption index of the extrudates [29]. According to Sereno et al., the higher water absorption of xanthan gum could be due to the formation of particles with swelling polyelectrolyte gel characteristics upon exposure to water. Cross linking of the helical structure of xanthan during extrusion processing could result in formation of such particles.

Water solubility index (WSI)

The water solubility index of fish feed indicates the integrity of extrudates in the aqueous environment. In general, the WAI and WSI values reflect the hydrophobic and hydrophilic nature of the extrudates which can be influenced by degree of starch degradation, protein denaturation, and the hydration dynamics of fiber. Typically, an inverse relation between WAI and WSI values would be anticipated for extrudates [30]. However, in our study, no such relation was observed for WAI and WSI values of gum included extrudates.

As gum inclusion level was increased from 6% to 10%, a striking increase could be observed in WSI values by 109.6%, 101.6%, 208.7%, 69%, and 116.4% for xanthan, guar, wheat gluten, CMC, and pullulan gum extrudates, respectively. The data showed a general trend toward higher WSI for the diets containing pullulan. In addition, the highest WSI value (28.43%) and the lowest WSI value (6.19%) were observed for pullulan and wheat gluten extrudates, respectively (Table 4). The higher WSI values for pullulan gum extruded diets might be due to extensive depolymerisation of pullulan exopolysaccharides and degradation of maltotriose units of pullulan in the mixture. On the other hand, the lower WSI values for wheat gluten extrudates could be due to interactions with gluten protein in the mixture which might also

Variable			Property								
G	SS (rpm)	GL (%)	UD (kg/m³)	BD(kg/m³)	ER(-)	WSI (%)	WAI(-)	PDI (%)	L (-)	a (-)	b (-)
Xanthan	100	3	856^{hi}	388^{g-l}	0.94^{c-f}	6.77^{j-l}	3.77^{f-i}	54.6^{mn}	46.9^{e-h}	5.04^{e}	22.5^{f-i}
	150		861^{hi}	384^{g-m}	0.98^{c-f}	7.60^{i-l}	3.79^{f-i}	56.4^{lm}	47.2^{e-h}	5.16^{de}	22.3^{f-i}
	100	6	1040^{b-g}	408^{gh}	0.90^{e-f}	9.52^{hi}	3.84^{e-i}	67.6^{g}	46.7^{e-h}	4.81^{ef}	21.4^{hi}
	150		913^{g-i}	401^{f-j}	0.99^{b-f}	7.08^{j-l}	4.27^{ef}	63.7^{h}	46.34^{e-h}	5.04^{e}	22.1^{ghi}
	100	10	1214^{a}	607^{a}	1.06^{a-d}	18.34^{c}	7.40^{bc}	90.1^{c}	50.5^{d}	7.50^{b}	25.4^{cd}
	150		1183^{ab}	575^{bc}	1.08^{a-c}	16.45^{d}	7.64^{ab}	97.4^{a}	53.3^{c}	8.40^{a}	29.6^{a}
Guar	100	3	854^{hi}	382^{h-m}	1.10^{a-c}	8.68^{ij}	4.00^{e-h}	54.0^{mn}	46.1^{f-h}	5.12^{de}	22.5^{f-i}
	150		986^{d-h}	368^{k-m}	1.15^{ab}	6.44^{l}	4.46^{e}	53.3^{n}	47.5^{e-g}	4.94^{ef}	22.3^{f-i}
	100	6	958^{e-i}	366^{k-m}	0.90^{e-f}	6.66^{j-l}	6.00^{d}	46.0^{o}	48.78^{de}	4.84^{ef}	22.1^{g-i}
	150		938^{f-i}	357^{m}	0.85^{f}	10.80^{gh}	5.57^{d}	44.6^{o}	50.7^{d}	4.92^{ef}	23.0^{e-g}
	100	10	1105^{a-e}	508^{de}	1.07^{abc}	21.20^{b}	8.00^{ab}	69.5^{g}	61.8^{a}	6.80^{c}	27.4^{b}
	150		1121^{a-d}	560^{c}	1.07^{abc}	14.04^{e}	8.10^{a}	81.1^{e}	46.6^{e-h}	6.97^{c}	24.1^{ef}
Wheat Gluten	100	3	1028^{b-g}	373^{j-m}	1.10^{abc}	7.74^{i-l}	2.64^{kl}	62.1^{hi}	47.6^{ef}	5.30^{de}	22.9^{e-h}
	150		$997d^{e-h}$	378^{i-m}	0.89^{ef}	7.13^{j-l}	2.64^{kl}	60.6^{ij}	45.4^{f-h}	4.93^{ef}	22.3^{f-i}
	100	6	923^{g-i}	361^{lm}	0.95^{c-f}	6.51^{kl}	$3.01j^{kl}$	58.6^{j-l}	45.1^{f-h}	5.07^{e}	21.5^{g-i}
	150		1120^{a-e}	369^{k-m}	0.90^{e-f}	6.19^{l}	3.03^{jk}	59.4^{jk}	44.6^{h}	5.07^{e}	22.2^{f-i}
	100	10	1162^{abc}	571^{c}	1.11^{abc}	19.66^{bc}	3.88^{e-i}	90.0^{c}	55.0^{c}	7.60^{b}	25.6^{c}
	150		1093^{a-f}	599^{ab}	1.22^{a}	19.48^{bc}	3.88^{e-i}	93.3^{b}	54.1^{c}	7.60^{b}	25.9^{c}
CMC	100	3	1004^{c-g}	422^{g}	0.94^{c-f}	7.79^{i-l}	3.78^{f-i}	79.6^{e}	41.4^{i}	4.80^{ef}	21.3^{hi}
	150		821^{i}	0.392^{h-k}	1.21^{a}	8.28^{i-l}	3.29^{ij}	72.7^{f}	45.1^{f-h}	5.14^{de}	22.5^{f-i}
	100	6	1045^{b-g}	391^{h-k}	0.99^{b-f}	7.59^{i-l}	4.25^{ef}	63.9^{h}	45.9^{f-h}	5.00^{ef}	22.5^{f-i}
	150		967^{d-i}	364^{k-m}	0.99^{b-f}	8.62^{i-k}	3.39^{h-j}	57.7^{kl}	46.2^{e-h}	4.86^{ef}	22.1^{g-i}
	100	10	1162^{abc}	524^{d}	1.07^{abc}	14.23^{e}	7.56^{ab}	74.9^{f}	58.8^{b}	7.60^{b}	28.8^{a}
	150		1210^{a}	563^{c}	1.03^{b-e}	13.24^{ef}	6.86^{c}	80.4^{e}	59.5^{b}	7.60^{b}	27.4^{b}
Pullulan	100	3	963^{d-i}	389^{f-j}	0.89^{e-f}	11.01^{gh}	2.59^{kl}	80.8^{e}	45.6^{f-h}	5.30^{de}	23.1^{e-g}
	150		911^{g-i}	406^{g-i}	0.88^{ef}	11.74^{fg}	2.64^{kl}	80.7^{e}	45.0^{f-h}	5.30^{de}	22.3^{f-i}
	100	6	927^{g-i}	412^{gh}	0.90^{e-f}	13.12^{ef}	2.38^{kl}	87.3^{d}	44.9^{gh}	5.6^{d}	23.7^{ef}
	150		1058^{a-g}	465^{f}	1.03^{b-e}	12.50^{e-g}	2.33^{l}	88.3^{cd}	45.0^{f-h}	5.11^{de}	21.2^{i}
	100	10	1179^{ab}	497^{e}	1.09^{abc}	27.06^{a}	4.11^{e-g}	96.2^{a}	53.6^{c}	5.20^{e}	17.4^{j}
	150		1064^{a-g}	500^{de}	1.19^{a}	28.43^{a}	3.48^{g-j}	95.2^{ab}	54.0^{c}	4.53^{f}	14.1^{k}

Table 5: Treatment combination effect of gum source (G), gum inclusion level (GL), and screw speed (SS) on extrudates' properties; means followed by similar letters for a given dependent variable are not significantly different at $P < 0.05$ among treatments.

Gum source	Interactions	Extrudate properties								
		UD(kg/m^3)	BD(kg/m^3)	ER(-)	WAI(-)	WSI (%)	PDI(%)	L(-)	a(-)	b(-)
Xanthan	GL	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	SS	0.2651	<.0001	<.0001	0.2601	0.1578	0.003	0.1671	<.0001	0.0004
	GL×SS	0.4714	<.0001	0.009	0.6844	0.2265	<.0001	0.1420	0.0002	0.0002
Guar	GL	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	SS	0.0789	0.0020	0.9732	0.9031	0.0133	<.0001	<.0001	0.7996	0.0332
	GL×SS	0.0357	<.0001	0.1278	0.4367	0.0003	<.0001	<.0001	0.4811	0.0010
Wheat gluten	GL	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	SS	0.1440	<.0001	0.0002	0.9397	0.0342	0.2877	0.0134	0.1836	0.5972
	GL×SS	<.0001	<.0001	<.0001	0.969	0.4662	0.0688	0.2619	0.2117	0.1122
CMC	GL	0.0074	<.0001	0.6944	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	SS	0.2902	0.1438	0.4042	0.0010	0.7023	0.0432	0.0171	0.7376	0.6711
	GL×SS	0.3678	<.0001	0.2669	0.4852	0.2218	0.0010	0.0556	0.5752	0.0792
Pullulan	GL	<.0001	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	0.0143	<.0001
	SS	0.5034	0.1845	0.0001	0.1155	0.4198	0.9184	0.9883	0.0105	0.0008
	GL×SS	<.0001	0.3666	0.0010	0.0952	0.401	0.1295	0.8807	0.1444	0.1416

Table 6: Interaction effects of extrudate properties due to source of gums, screw speed and gum levels (p values).

contribute to decreasing the leaching of the ingredients in the sample mixture. It can be also due to protein denaturation and increased hydrophobic amino acid side chain exposure to water.

Changing the screw speed did not produce any significant differences for WAI and WSI values of the gum treated extrudates at α=0.05 (Table 3). Also, the interaction effect of GL×SS was not significant for WAI and WSI values (Table 6).

In a study conducted by Rusco et al., the effects of CMC, carrageenan, agar, and gelatin binders and feed moisture content on pelleted crayfish feed were investigated [31]. They reported that carrageenan and CMC binders were remarkably more effective in maintaining water stability. They also suggested that alginate at low moisture content of 10% resulted in more water stable pellets. In our experiment, CMC inclusion resulted in extrudates with relatively low water solubility index (mean=9.95%; CV=28%). Kaur et al. postulated that the water absorption capacity of extruded products increased with an increasing concentration of polysaccharides of the blend, however this response strongly depended on the type of polysaccharides [32]. A lower water solubility index provides higher water stability of the extrudates, and increases feed availability duration for fish, and provides less or slower nutrient losses. However, very low water solubility may result in lower digestibility of the extruded feed. When the water solubility and stability are optimized, there will likely be maximum nutrient utilization (of a given blend) which enhances the feed efficiency.

Pellet durability index (PDI)

Not only is the water stability of fish feed extrudates is important to aquaculture industry, but the physical stability of these products against the external mechanical forces during the bulk handling and storage processes is also a very crucial factor [33]. One method to quantify the mechanical strength of fish feed extrudates is by determining the pellet durability index (PDI) of extrudate or pellet. As shown in Table 2, the PDI values of the extrudates were significantly affected by type of gums. However, the PDI values for xanthan (mean=71.6; CV=1.37), wheat gluten (mean= 70.6; CV=2.27) and CMC (mean: 71.5; CV=3.33) extrudates were not significantly different (α=0.05). The main effects of independent extrusion variables on the PDI of the gum-treated products are presented in Table 3. While changing screw speed did not significantly (α=0.05) affect the PDI values of extrudates, varying the inclusion level of gum exhibited a significant effect on this property. Increasing GL from 3% to 10%, curvilinearly increased PDI values for xanthan, guar, and wheat gluten extrudates. Increasing GL from 3% to 10% linearly (R^2=0.97) increased PDI values for pullulan extrudates, but it did not change this value for CMC extrudate. This rising trend in PDI could be due to the fact that the higher concentration of gums resulted in more contact surfaces among the extrudates particles (by reducing void spaces) leading to a chemical reaction during the extrusion cooking, transformation of ingredients' nature, and increasing the pellet durability [34].

The data showed a general trend toward higher PDI for the diets containing pullulan, ranging from 80.7% to 96.2%, as depicted in Table 4. The higher PDI values of pullulan gum extrudates indicate that these extrudates could be more resistant to mechanical forces during transportation and storage.

According to Rosentrater et al., PDI quality of the extrudates can be influenced by the extent of heat treatment, along with the level of macromolecules transformed (such as starch gelatinization) and water content applied during the extrusion process. In fact, the gums used in this study came from different sources. This is relevant, as the differing constituent compositions of gums may interact with other ingredients of the blend. Hence, the possibility of multifactorial interpretation for some of the effects cannot be ignored. Also, the striking effect of gum type on PDI values of the fish feed extrudates might be due to the differing behaviors of these non-starch polysaccharides in gelatinization phenomena during the extrusion processing. Although, non-starch polysaccharides do not contribute in gelatinization, their presence in feed matrix can generally alter the gelatinization characteristics of the starch component [35]. Consequently, gums can affect the rheology of the extruding blend, viscosity, developed barrel pressure and ultimately the extent of heat treatment.

The lower PDI values of guar gum extrudates might be due to the interaction of its polysaccharides, galactose and mannose, with the proteins, which resulted in poor macromolecular transformation, weak gelation, reduced cohesion, and hence the durability of the extrudates. Brinker et al. suggested that addition of non-polysaccharide binder such as guar gum in plant-based fish diets could successfully suppress the mechanical instability of chyme and feces. However, they did not study the effect of this non-starch polysaccharide on mechanical stability of fish feed before consumption. As depicted in Table 6, interaction effect of GL×SS on PDI was not significant for extrudates containing pullulan and wheat gluten.

Color (L, a, and b)

Color change is one of the quality indicators for protein-based cooked materials in the food and feed industries. The interaction between the reducing ends of carbohydrate and protein components of the feed can lead to a Maillard reaction, resulting in a wide range of products with significantly different nutritional values. These changes can decrease digestibility and bioavailability of essential amino acids such as lysine. Statistical analyses across all the collected data confirmed that changes in type of gum did not result in statistically significant differences in color values of extrudates at $\alpha=0.05$ (Table 2). Only the yellowness score of the pullulan extrudates was considerably different compared to that of the other extrudates (Table 2). For all gums used in this study, lighter extrudates resulted in greater PDI values, except for guar gum (Table 6).

Conclusions

The objective of this study was to assess and compare the effects of non-starch polysaccharide gums including plant-origin gum (i.e. guar, wheat gluten, and CMC) and microbial-origin exopolysaccharides (i.e. xanthan and pullulan) on physical properties of aquadiet during single-screw extrusion processing. Using the extrusion conditions employed during these experiments it was found that exopolysaccharide gums resulted in extrudates with significantly better PDI than plant-origin gums. The water solubility index of pullulan included extrudates was considerably high compared to the other products and it constantly increased with increasing level of gum inclusion. It was also observed that the mean values of expansion ratio for the diets containing exopolysaccharide gums (i.e. xanthan and pullulan) were smaller than those of the other diets. However, at the highest levels of gum inclusion and screw speed, both pullulan and wheat gluten gums showed to have better contribution in expandability of the fish feed extrudates.

Inclusion of gums at the highest level resulted in denser extrudates; it also simultaneously induced an increase in expansion ratio of all products. The yellowness score of pullulan extrudates at higher level of gum inclusion was considerably low compared to other extrudates which might result in differences in nutritional quality of the extruded feeds. It is noteworthy that the extrudates' textural variability was reduced by higher levels of gum additions, resulting in more uniform products.

Future research could involve testing the effect of gradual substitution of starch with non-starch polysaccharides, the effect of feed composition, as well as the effect of different extrusion processing parameters on physical quality of plant-based fish feed extrudates.

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