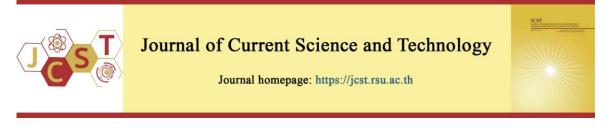
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Quality improvement of plant-based patty using methylcellulose, κ-carrageenan and xanthan gum

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Abstract

Nowadays, people are more health-conscious and interested in consuming plant-based products in preference to processed meat that contains high cholesterol and unhealthy fats. Production of good quality meat alternatives is urgently required. In the food industry, hydrocolloids have been used to improve product quality. The objective of this study was to use hydrocolloids including methylcellulose (MC), κ -carrageenan (κ -CGN) and xanthan gum (Xan) (0.1%, 0.2% and 0.3% w/w) to improve the quality of plant-based patties formulated by textured soy protein (TSP). Plant-based patties containing MC exhibited higher (p<0.05) hardness, springiness, cohesiveness and chewiness values than plant-based patties containing κ -CGN and Xan. Hardness, chewiness and shear force increased with increasing MC and κ -CGN concentrations but decreased with increasing Xan concentration. Moisture content, color and sensory attributes (appearance, color and soy flavor) of plant-based patty were not significantly affected by the different types of hydrocolloids. Addition of κ -CGN (0.1% and 0.2%) gave the highest taste, texture and higher overall liking scores, followed by MC and Xan (p<0.05). Results indicated that hydrocolloids could be applied in the food industry to enhance the quality of plant-based products.

Keywords: plant-based patty; consumer acceptance; hydrocolloids; shear force; textured soy protein; texture profile analysis

1. Introduction

Modern consumers have heightened awareness and concern about environmental impacts and health care, with increasing numbers now choosing plant-based meat analog products (also termed meat alternative, vegan or vegetarian meat), which are mainly made from plant-based proteins (Aschemann-Witzel, Gantriis, Fraga, & Perez-Cueto, 2020). Protein overconsumption from animal sources containing high cholesterol and unhealthy fats increases the risk of cardiovascular disease and other related afflictions such as cancer and diabetes (Boada, Henríquez-Hernández, & Luzardo, 2016; Dominguez et al., 2018; Nadathur, Wanasundara, & Scanlin, 2017).

Various plant protein sources include cereals (wheat, corn, rice), legumes (soy, beans, peas), oil seeds (sunflower, flaxseed, sesame seed), nuts (almond, peanut, cashew) and vegetables (Nasrabadi, Doost, & Mezzenga, 2021). Many forward-looking companies have developed various plant proteins with meat-like structural, functional, sensorial and textural characteristics using processing technologies such as extrusion, spinning and simple shear flow (Kyriakopoulou, Dekkers, & van der Goot, 2018). Most protein products are made from defatted soy flour, soy protein concentrate and soy protein isolate, known as textured soy protein (TSP). Soybeans are used as ingredients in meat analogs because they are abundant, low cost and have suitable textural, nutritional and functional properties (Kyriakopoulou et al., 2018; Kyriakopoulou, Keppler, & van der Goot, 2021; Riaz, 2011). Textured soy protein and hydrocolloids have been used to improve the quality of plant-based patty.

Hydrocolloids are polysaccharides and proteins that enhance the viscosity, thickening, gelling agents, binders and stability of food products (Willams, & Phillips, 2009; McArdle, & Hamill, 2011; Razavi, 2019). Xanthan gum (Xan) is an anionic microbial heteropolysaccharide produced by Xanthomonas campestris. Xan is distinguished by its excellent stability in thermal and acid systems (Pongsawatmanit, Chantaro, & Nishinari, 2013; Wang et al., 2016). Xan can reduce cooking loss and hardness of meat products (Zhao, Wang, Li, & Zhou, 2021). Methylcellulose (MC) and carrageenan improve textural properties and binding abilities, providing the desirable gelling and thickening of meat products (Kyriakopoulou et al., 2021). In high moisture extrusion technology, soya protein concentrate blended with iotacarrageenan significantly increased the textural properties (cutting force and elasticity) and sensorial properties (such as hardness and fibrous structures) (Palanisamy, meat-like Töpfl. Aganovic, & Berger, 2018). Previous research has shown that addition of hydrocolloids improves the quality of beef patties (Kilincceker, & Yilmaz, 2016; Pematilleke, Kaur, Wai, Adhikari, & Torley, 2021). Carrageenan addition increased water binding capacity and improved the textural property of turkey meat sausages (Ayadi, Kechaou, Makni, & Attia, 2009), while addition of κ-carrageenan (κ-CGN) improved the textural and gel properties of frankfurters but this depended on k-carrageenan incorporation methods (ĸ-CGN powder, ĸ-CGN water suspension and κ -CGN brine suspension) (Cao et al., 2021). Bakhsh et al. (2021) reported that adding MC improved the quality of plant-based meat patty. However, a knowledge gap exists as to how hydrocolloids affect the quality of plant-based meat products.

2. Objective

The objective of this study was to investigate the effect of methylcellulose, κ -carrageenan and xanthan gum on the quality of plant-based patty.

3. Materials and methods

3.1 Materials

Textured soy protein (TSP) was obtained from The Solae Company, Geneva, Switzerland. The hydrocolloids used for the experiment were methylcellulose (MC), κ -carrageenan (κ -CGN) and xanthan gum (Xan). These were sourced from Ashland Global Specialty Chemicals Inc. (Delaware, USA), Marcel Carrageenan (Quezon, Philippines) and C.E. Roeper GmbH (Hamburg, Germany), respectively. Isolated soy protein, pea fiber, yeast extract and beetroot powder were obtained from PROFAM-974, ADM, Chicago, USA, J. Rettenmaier & Söhne GmbH & Co. KG, Rosenberg, Germany, Lallemand Inc., Montréal, Canada and Naturex AG, Bischofszell, Switzerland, respectively. Seasoning was purchased from a local supermarket in Bangkok, Thailand. All ingredients used in this study were of food-grade purity.

3.2 Preparation of plant-based patty

Three hydrocolloids, MC, κ -CGN and Xan at three concentrations of 0.1%, 0.2% and 0.3% were used in the experiment. For hydrocolloid dispersion, each hydrocolloid was added to a mixture of salt and sucrose in water and stirred continuously for 30 min using a magnetic stirrer.

Plant-based patties (1,500 g) were prepared for each formulation. The TSP was soaked in water (ratio of 1:4 w/v) at 80±5°C for 20 min, rinsed with cold water and then shaken to remove excess water using a food processor (Panasonic, MK-F300WSN, Thailand) for 40 sec and refrigerated until the temperature reached 0-4°C. Subsequently, cold TSP (55% w/w in formulation) was mixed with isolated soy protein, pea fiber, yeast extract, beetroot powder and seasoning for 5 min using a bowl lift stand mixer (Kitchen Aid, Classic Plus Stand Mixer, StJoseph, MI, USA). The hydrocolloid dispersion was then added to the mixture and stirred continuously for 2 min before adding small pieces of solid coconut oil and mixing continuously again for 1 min. Subsequently, 42 ± 1 g of the mixture was shaped into a cylindrical patty $(60\pm1 \text{ mm diameter and } 15\pm1 \text{ mm in height})$ using a patty press maker. Finally, plant-based patties were frozen at -18°C for 30 min and then steamroasted at 160±2°C for 3 min using a convection oven (Combi Convotherm OES6.10, Convotherm Elektrogeräte GmbH, Germany). The plant-based patties were vacuum-packed and stored at -18±2°C.

3.3 Moisture content and color measurement

After warming in a commercial microwave (Panasonic, NE-1353, Thailand) at 1,300W for 90 sec, the plant-based patties were analyzed for moisture content and color. To analyze moisture content, the plant-based patties were cut into small pieces. Moisture content was evaluated

using a hot air oven (LDO-030E, Daihan LabTech Co., Ltd., Kyonggi-Do, Korea) at 105°C until constant weight (AOAC, 2000).

Color measurements as surface color values of cooked plant-based patties were determined using a colorimeter (CR-10 Plus, Konica Minolta, Osaka, Japan) with standard illuminant D65, observer 10°, 8 mm diameter aperture and 8° viewing angle. The CIE color values were reported as L* (varying from 0 to 100 = black to white), a* displaying the brightness value of color (-a* and +a* = greenness and redness) and b* (-b* and +b* = blueness and yellowness) (Ly, Dyer, Feig, Chien, & Del Bino, 2020). Color measurements were recorded in triplicate at three positions on each patty. Results were expressed as mean values.

3.4 Texture profile analysis and shear force

Texture profile analysis (TPA) and shear force (maximum shear force) were evaluated using a Texture Analyzer (TA-XT plus, Stable Micro System Ltd, UK) equipped with a 50 kg loading cell. The cylindrical samples (25±1 mm diameter and 15±1 mm in height) were warmed using the commercial microwave (Panasonic, NE-1353, Thailand) at 1,300W for 30 sec. All samples were controlled temperature at 50-55°C by food warmer (LR-WS-809L, Linkrich Enterprise Limited, China) before measurement. For TPA, each cylindrical sample was double-cycle compressed with 50% deformation using pretest, test and posttest speed of 2 mm/sec using a 50-mm diameter probe (P/50). Thirty samples were analyzed to obtain an average value and standard deviation for all TPA parameters. Force time deformation curves were used to calculate hardness (maximum force on compression cvcle). the first springiness. cohesiveness and chewiness following the method described by Bourne (2002). For shear force, each cylindrical sample was sheared at 2 mm/sec speed using a Warner-Bratzler shear fixture (60° Vshaped blade). Shear force results were expressed as mean values.

3.5 Sensory evaluation

Sensory evaluation of plant-based patty was assessed by 100 panelists (students and employees at Panyapiwat Institute of Management, Thailand). Samples were warmed in a commercial microwave (Panasonic, NE-1353, Thailand) at 1,300W for 90 sec, divided into six equal pieces, and coded with three-digit random numbers to avoid bias before serving to the panelists at 50-55°C. Unflavored crackers and water were provided to rinse the mouth between sample testing. Attributes of appearance, color, soy flavor, taste, texture, overall liking and consumer acceptance were evaluated using a 9-point hedonic scale from 1 (extremely dislike) to 9 (extremely like). All samples were assigned for sensory evaluation at the same time. Informed consent was obtained from all panelists. This study was approved by the Human Ethics Research Panyapiwat Institute of Management (PIM-REC 013/2020).

3.6 Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics 24 software, with results presented as mean values \pm standard deviation. All data were subjected to two-way analysis of variance (two-way ANOVA) with two factors: (Factor 1, types of hydrocolloids and Factor 2, concentrations of hydrocolloids). Duncan's multiple range test was applied to determine differences at the p<0.05 level of significance. All experiments were conducted in triplicate. Pearson's correlation matrix was used to evaluate the hydrocolloid concentrations and parameters of hardness, springiness, cohesiveness, chewiness and shear force of plant-based patty containing different hydrocolloids (MC, κ -CGN and Xan).

4. Results and discussion

4.1 Moisture content and color of plant-based patties

The color of cooked plant-based patties was measured by the CIE (L*a*b*) system, as shown in Table 1. Results indicated that the color of all cooked samples was not significantly different (p>0.05), while addition of different hydrocolloids (MC, κ -CGN and Xan) had no visual impact. Concentrations of added hydrocolloids (0.1%, 0.2% and 0.3%) were low, with only slight variations in color. These findings concurred with Palanisamy, Töpfl, Aganovic, and Berger (2018) who investigated the effect of iota-carrageenan addition on the properties of soya protein concentrate used for meat analogs. Moisture contents of the plantbased patties after warming containing MC, κ -CGN and Xan as shown in Table 1.

Hydrocolloid	Concentration (%)	L ^{* ns}	a ^{* ns}	b* ns	Moisture content (%wb)
MC	0.1	40.63±1.61	16.78±1.00	17.56±0.79	61.47±1.97 ^{ab}
	0.2	41.36±2.21	16.39±0.11	17.89±2.35	63.30±1.01 ^a
	0.3	42.31±2.70	16.01±0.22	16.64 ± 0.40	60.53±1.71 ^b
κ-CGN	0.1	40.50±1.59	16.45±0.29	16.97±0.23	62.35±1.62 ^{ab}
	0.2	41.58±2.20	15.99±0.02	17.16±0.13	64.05±1.23 ^a
	0.3	40.02 ± 2.57	16.19±0.36	17.22±0.97	62.11±1.17 ^{ab}
Xan	0.1	43.63±3.60	15.49 ± 0.30	16.56±1.07	63.90±1.05 ^a
	0.2	44.21±2.65	16.42±0.13	16.07±0.27	64.16±0.78 ^{ab}
	0.3	42.68±1.24	16.08 ± 0.42	16.35±0.13	61.85 ± 1.54^{a}

Table 1 Color and moisture content of plant-based patties with different hydrocolloids (MC, κ -CGN and Xan) and concentrations (0.1%, 0.2% and 0.3%)

Mean \pm standard deviation values (n=3) followed by different lower case superscripts within the same column are significantly different (p<0.05) by Duncan's multiple range test.

^{ns} no significant differences at the 5% level.

4.2 Texture profile analysis and shear force

Textural properties are important for developing plant-based patties. Two-way ANOVA results showed that both types and concentrations of hydrocolloids, as well as their interaction, had a significant effect on hardness, springiness, cohesiveness, chewiness and shear force (p<0.05) (Table 2). When comparing different types of hydrocolloids, plant-based patties containing MC showed the highest hardness, springiness, cohesiveness and chewiness followed by ĸ-CGN and Xan, respectively (p<0.05) (Table 3). Our results concurred with Pematilleke, Kaur, Wai, Adhikari, and Torley (2021) who reported that hardness and cohesiveness of beef patties prepared with ĸ-CGN were higher than Xan. Hardness, springiness, cohesiveness and chewiness of plantbased patties increased with increasing MC concentration (p<0.05). Previous studies reported that increasing concentrations of MC proportionally increased the hardness and chewiness of plantbased meat (Bakhsh et al., 2021; Sakai, Sato,

Okada, & Yamaguchi, 2021). Increase in κ-CGN concentration from 0.1% to 0.3% increased hardness and chewiness of all patties but decreased springiness and cohesiveness (p<0.05). Avadi, et al. (2009) and Arora, Kamal, & Sharma (2016) reported that hardness and chewiness increased with increasing CGN concentration. By contrast, hardness of plant-based patties decreased with increasing Xan concentration (p<0.05). No significant differences (p>0.05) were recorded in springiness, cohesiveness and chewiness at different concentrations. Our results agreed with Majzoobi, Talebanfar, Eskandari, and Farahnaky (2017) who reported that addition of Xan reduced hardness of meat-free sausages. Rather et al. (2015) reported that hardness decreased with increasing Xan in goshtaba (a traditional meat product of India). Moisture content of plant-based patties after warming containing 0.3% MC (Table 1) was lowest and gave the highest values of hardness, springiness, cohesiveness and chewiness (Table 3).

Table 2 Two-way ANOVA of textural properties of plant-based patties with different hydrocolloids (MC, κ -CGN and Xan) and concentrations (0.1%, 0.2% and 0.3%)

	Main effec	rt			Interaction		
Quality	Hydroc	colloid	Concent	ration	Hydrocolloid x Concentratio		
	F-value	p-value	F-value	p-value	F-value	p-value	
Hardness	804.75	< 0.01	24.91	< 0.01	30.406	< 0.01	
Springiness	518.68	< 0.01	0.42	0.66	4.383	< 0.01	
Cohesiveness	531.67	< 0.01	4.54	< 0.01	8.132	< 0.01	
Chewiness	834.92	< 0.01	35.29	< 0.01	37.043	< 0.01	
Shear force	36.97	< 0.01	2.20	0.11	7.017	< 0.01	

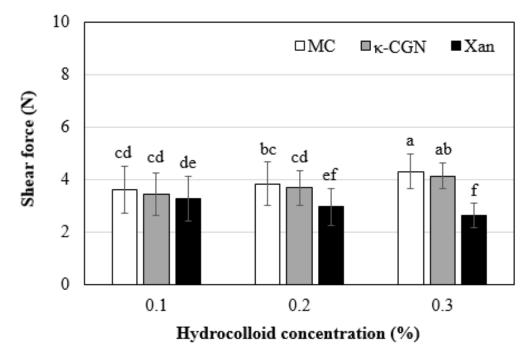
Table 3 Textural p	properties of	plant-based	patties p	roduced wi	th different	hydrocolloids	and concentrations
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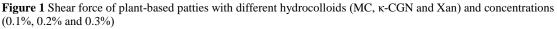
Hydrocolloid	Concentration (%)	Hardness (N)	Springiness (-)	Cohesiveness (-)	Chewiness (N)
MC	0.1	6.13±0.77°	0.59 ± 0.07^{b}	0.38±0.03 ^b	1.40±0.31°
	0.2	6.88±1.22 ^b	0.63±0.07 ^{ab}	0.39±0.04 ^b	1.70±0.43 ^b
	0.3	8.41 ± 0.85^{a}	0.66 ± 0.05^{a}	0.43 ± 0.05^{a}	2.40±0.35 ^a

Hydrocolloid	Concentration (%)	Hardness (N)	Springiness (-)	Cohesiveness (-)	Chewiness (N)
κ-CGN	0.1	5.12±0.49 ^e	0.50±0.08°	0.36±0.03°	0.92±0.21 ^d
	0.2	5.27±0.82e	$0.48\pm0.08^{\circ}$	0.35±0.03°	0.90 ± 0.30^{d}
	0.3	5.72 ± 0.72^{d}	$0.47 \pm 0.07^{\circ}$	0.35±0.03°	0.95 ± 0.32^{d}
Xan	0.1	3.12 ± 0.60^{f}	0.29 ± 0.07^{d}	0.25 ± 0.02^{d}	0.23±0.09 ^e
	0.2	2.69±0.49g	0.26 ± 0.09^{d}	0.24 ± 0.02^{d}	0.17±0.07 ^e
	0.3	2.45 ± 0.30^{g}	0.27 ± 0.07^{d}	0.25 ± 0.02^{d}	0.16±0.07 ^e

Mean \pm standard deviation values (n=30) followed by different lower case superscripts within the same column are significantly different (p<0.05) by Duncan's multiple range test.

Tenderness was recorded as the maximum force required to cut through the sample crosssection, called shear force. The shear force increased significantly at 0.3% concentration with increasing MC and κ -CGN contents (Figure 1), whereas it decreased significantly at 0.3% with increasing Xan concentration (p<0.05). Our results agreed with Zhao, Wang, Li, and Zhou (2021) who reported that addition of Xan reduced shear force of beef.





Pearson's correlation coefficient (r) was applied to quantify the relationship between hydrocolloid concentration, hardness, springiness, cohesiveness, chewiness and shear force for cooked plant-based patties produced with different hydrocolloids (MC, κ -CGN and Xan). Concentration of MC had positive correlation with hardness (0.694), springiness (0.409), cohesiveness (0.435), chewiness (0.739) and shear force (0.335) (Table 4, p<0.01), while concentration of κ -CGN gave a positive correlation for hardness (0.341) and shear force (0.392, p<0.01). Concentration of Xan had a negative correlation with hardness (0.499), chewiness (0.341) and shear force (0.371) (p<0.01). Results indicated that addition of hydrocolloids improved the textural properties of plant-based patties, especially hardness, chewiness and shear force parameters.

	МС				к-CGN				Xan						
	Conc entr ation	Ha rd nes s	Spr ingi ness	Coh esiv enes s	Ch ewi nes s	Conc entr ation	Ha rd nes s	Spr ingi ness	Coh esiv enes s	Ch ewi nes s	Conc entr ation	Ha rd nes s	Spr ingi ness	Coh esiv enes s	Ch ewi nes s
Con															
centr						1.00					1.00				
ation	1.000					0					0				
											-				
Hard	0.694	1.0				0.34	1.0				0.49	1.0			
ness	**	00				1**	00				9**	00			
Spri						-	0.2				-				
ngin	0.409	0.1	1.00			0.14	79	1.00			0.14	0.1	1.00		
ess	**	78	0			5	**	0			5	85	0		
Coh						-	0.2				-				
esive	0.435	0.1	0.62	1.00		0.17	38	0.42	1.00		0.05	0.1	0.52	1.00	
ness	**	90	6**	0		5	*	8**	0		7	52	7**	0	
Che		0.7					0.7				-	0.6			
wine	0.739	43	0.68	0.74	1.0	0.05	09	0.79	0.67	1.0	0.34	28	0.84	0.61	1.0
SS	**	**	8**	7**	00	4	**	6**	3**	00	1**	**	9**	0**	00
Shea		0.2	-	-	0.3			_	-	-	-		-	-	
r	0.335	99	0.26	0.34	91*	0.39	0.0	0.09	0.19	0.0	0.37	0.0	0.15	0.02	0.1
force	**	*	8*	5**	*	2**	53	2	8	79	1**	97	0	9	19

 Table 4 Correlation matrix between hydrocolloid concentration, TPA parameters and shear force of plant-based patties with different hydrocolloids

*Correlation is significant at the 0.05 level (2-tailed), p<0.05.

** Correlation is significant at the 0.01 level (2-tailed), p<0.01.

4.3 Sensory evaluation

Sensory evaluation of plant-based patties containing TSP mixed with different hydrocolloids (MC, κ -CGN and Xan) and concentrations (0.1%, 0.2% and 0.3%) was assessed by a hedonic test, as shown in Table 5. Appearance, color and soy flavor of all samples were not significantly (p>0.05) affected by addition of hydrocolloids as a clear, colorless and flavorless liquid. Color-based sensory evaluation results corresponded to the statistical test results of sample color values measured by the color reader. Significant (p<0.05) differences were recorded between taste, texture and overall liking of the samples. Addition of κ -CGN improved taste, texture and overall liking scores more than MC and Xan, while Xan had a higher viscosity than κ -CGN and MC, resulting in softer texture of plant-based patties. Milani, and Maleki (2012) reported that type and amount of hydrocolloid impacted functional food properties such as thickening, gelling, stabilization and coating. Both texture and viscosity affect sensory properties in food products. Therefore, hydrocolloids should be used in small amounts as food additives. More than 70% acceptance was recorded by the panelists for plant-based patties mixed with 0.1%-0.3% κ -CGN and 0.3% MC (Figure 2).

Table 5 Sensory evaluation of plant-based patties produced with different hydrocolloids and concentrations

Hydrocolloid	Concentration	Attributes								
Hydrocolloid	(%)	Appearancens	Color ^{ns}	Soy flavor ^{ns}	Taste	Texture	Overall liking			
MC	0.1	6.13±1.55	6.29±1.50	6.06 ± 1.62	5.87 ± 1.76^{d}	5.56±1.97 ^e	5.83±1.64 ^d			
	0.2	6.06 ± 1.56	6.26±1.51	5.93±1.59	6.24±1.64°	5.98±1.75°	6.08±1.72°			
	0.3	6.39±1.52	6.28 ± 1.58	6.13±1.46	5.93±1.60 ^d	5.80 ± 1.85^{d}	6.25±1.59 ^b			
κ-CGN	0.1	6.41±1.40	6.46±1.41	6.25 ± 1.67	6.54 ± 1.68^{a}	6.36±1.77 ^b	6.53±1.62 ^a			
	0.2	6.29±1.61	6.40 ± 1.56	6.17±1.64	6.43±1.51 ^b	6.52±1.63 ^a	6.55±1.35 ^a			
	0.3	6.06 ± 1.76	6.28±1.66	6.14±1.65	6.25±1.56°	5.98±1.78°	6.26±1.33 ^b			
Xan	0.1	6.10±1.62	6.13±1.61	5.82 ± 1.69	5.76±1.76 ^e	5.46±1.82 ^e	5.72±1.52 ^e			
	0.2	6.21±1.75	6.14±1.61	6.21±1.63	5.91±1.72 ^d	5.30 ± 2.01^{f}	5.83±1.74 ^d			
	0.3	6.32±1.69	6.47±1.47	6.08 ± 1.50	5.49 ± 1.64^{f}	5.08 ± 1.84^{g}	5.62±1.59 ^e			

Mean \pm standard deviation values followed by different lower case superscripts within the same column are significantly different (p<0.05) by Duncan's multiple range test.

^{ns} no significant differences at the 5% level.

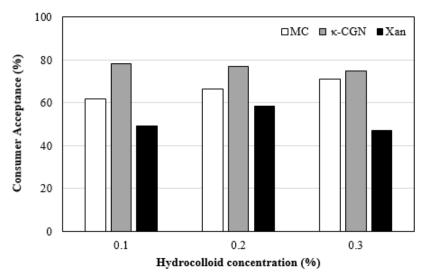


Figure 2 Consumer acceptance of plant-based patties produced with different hydrocolloids (MC, κ -CGN and Xan) and concentrations (0.1%, 0.2% and 0.3%)

5. Conclusions

Moisture content, color measurement, textural and sensory properties of plant-based patties with textured soy protein (TSP) were assessed after incorporation of three hydrocolloids (MC, κ -CGN and Xan) at concentrations of 0.1%, 0.2% and 0.3%. Addition of both hydrocolloid type and concentration significantly affected the quality characteristics of plant-based patties, except for the color value. Increasing MC concentration displayed significant increases in all texture profile and shear force values, while increasing K-CGN concentration displayed significant increases in hardness and shear force, and increasing Xan concentration showed significant decreases in hardness and shear force. At the same concentration, plant-based patties containing 0.3% MC had the lowest moisture compared to ĸ-CGN and Xan. These patties had resistance during chewing, while plantbased patties containing Xan were soft and mushy. These results concurred with the texture profile analyses for hardness, springiness, cohesiveness, chewiness and shear force. Addition of MC and k-CGN increased hardness, chewiness and shear force of plant-based patties but these properties decreased when adding Xan. For sensory evaluation, addition of κ -CGN gave the highest taste, texture and overall liking scores, followed by MC and Xan. More than 70% of the panelists accepted plant-based patties mixed with 0.1-0.3% K-CGN and 0.3% MC. Information gained in this study can be used to improve the quality of meat alternative products in various food industries.

6. Acknowledgements

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