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EFFECT OF CARBOXYMETHYL CELLULOSE (CMC) ON PERCEPTION OF ASTRINGENCY OF PHENOLIC COMPOUNDS

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The influence of carboxymethyl celluloses (CMCs) on astringency of tannic acid and polyphenol extracts obtained from black chokeberry fruits has been analysed. The study was performed with the critical concentrations (c^*) of CMCs as well as with values above and bellow c^*. The results were expressed as a percentage of unreduced astringency sensation. The ability of hydrocolloids with low (CMCLV), medium (CMCMV) and high viscosity (CMCHV) to mask the astringency was observed to be similar and to depend on their concentration. The highest reduction of astringency was above the c^* concentrations. Addition of CMCLV to solutions of sucrose or caffeine lowered the intensity of astringency generated by tannic acid. The results indicated that CMCLV masked also the astringency of polyphenols extracted from black chokeberry fruits.

INTRODUCTION

Hydrocolloids are widely used in the food industry as thickeners, stabilisers and gelling agents in many food products. There is currently much interest in hydrocolloids because their addition to food may modify perception and release the volatile as well as non-volatile compounds of flavour [Malone *et al*., 2003; Doyen *et al* 2001; Malkki *et al.,* 1990]. The effect of these polymers on the basic taste, especially on sweetness, has been studied extensively [Baines & Morris 1987; Paulus & Haas 1980; Pangborn, 1973]. However little reports have been found in literature on the effect of hydrocolloids on perception of astringency of non-nutrient bioactive compounds including polyphenols. It is well known that astringency might be a major problem if non-nutrient bioactive compounds were used as a functional ingredient, as the astringency would limit their consumption [Drewnowski & Gomez-Carneros, 2000]. Our preliminary study indicated that the astringency could be reduced by some food gums such as guar, xanthan, arabic and carboxymethyl cellulose (CMC). Among the investigated hydrocolloids the CMC, which is commonly used in food as a structure-forming substance, was the best astringency masker [Troszyńska *et al.,* 2007a]. The present study is an extension to our earlier studies regarding the ability of hydrocolloids to mask the astringency. The aim of this research was to explore the effect of three types of carboxymethyl celluloses (CMCs), *i.e.* low viscosity (CMCLV), medium viscosity (CMCMV) and high viscosity (CMCHV), on perception of astringency in a model system using tannic acid as a referential standard. The interactive effects of CMCLV and sucrose as well as caffeine in modifying astringency of tannic acid and polyphenol extracts from the black chokeberry (*Aronia melanocarpa Elliot*) fruits were evaluated as well.

MATERIAL AND METHODS

Material

Carboxymethyl celluloses (CMCs): low viscosity (CMCLV), medium viscosity (CMCMV), high viscosity (CMCHV), and tannic acid were all pure grade reagents purchased from Sigma – Aldrich Chemie Gmbh. The astringency sensation was represented by tannic acid and chokeberry polyphenols which were extracted from fruits of black chokeberry (*Aronia melanocarpa Elliot*) grown at an ecological farm in the north–eastern Poland. Four polyphenolic extracts prepared for the study were abbreviated as: CA – acetone extract of chokeberry; CA+CMCLV – acetone extract of chokeberry with CMCLV; CE – ethanolic extract of chokeberry, and CE+CMCLV – ethanolic extract of chokeberry with CMCLV.

Viscosity measurement

The apparent viscosity of CMCLV, CMCMV and CMCHV solutions was measured with a rotary viscometer Rheotest 2 Type RV2 (MLW, Germany). The tests were conducted at 20°C at a shear rate of 240.57 s^{-1}. The concentration *versus* viscosity plot was calculated for each type of CMCs. On the basis of the obtained curves the critical values (coil overlap value – c^*) for particular hydrocolloids were computed according to Cook *et al.* [2002]. The mode of c^* calculation is shown in Figure 1. The results are means of three replications.

Chemical analyses

Two spectrophotometric methods were used in this study. In the first one, tannins of the experimental extracts (CA, CA+CMCLV, CE and CE+CMCLV) were evaluated by

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means of the protein precipitate assay according to Hagerman & Butler [1978]. Proteins: bovine serum albumin (BSA) and human salivary protein (HSPs) were both used in the investigation. BSA (fraction V, fatty acid free) was purchased from Sigma Chemical Co. and HSPs was obtained from five healthy, non-smoking panellists [Troszyńska *et al*., 2006]. The results are expressed as absorbance values (A_{510}) per gram of extracts.

In the second method the content of anthocyanins in the extracts was estimated according to Wrolstad [1976]. The data were calculated on cyanidin-3-5-diglucoside equivalent per gram of sample (repetition n=4).

Sensory evaluation

A sensory panel consisted of nine members previously selected and trained according to ISO guidelines [PN-ISO 8586-2:1994]. Four of them were familiarised with the astringency evaluation by participation in previous related study [Troszyńska *et al*., 2006; 2007a,b]. One formal session was preceded by training sessions to familiarise the panelists with the masking concept of astringency.

The ability of CMCLV, CMCMV and CMCHV to reduce the astringency was evaluated using the method of taste indicator [Katsuragi *et al*., 1997]. From standard (tannic acid) five aqueous solutions in the range of 0.0, 0.05, 0.10, 0,15, and 0.2 have been prepared from a 2% stock solution in redistilled water. For testing 10-mL volume individual samples were prepared, coded and presented in random to each panel member. The subject was requested to compare taste intensity of a test solution (CMC with tannic acid) with that of the standard solution and to select the standard solution with a taste intensity equivalent to that of the given test solution. The results were expressed as a percentage of unreduced sensation. As astringency is quite a persistent sensation, a 3-min break was taken between the samples, during which the panellists were asked to eat unsalted biscuits as a neutraliser and rinse their mouths thoroughly with spring water.

The effect of CMCLV at c* concentration on the astringency generated by tannic acid (0.2% w/v) was determined with the method of sensory scaling [PN-ISO 4121:1987] at various concentrations of sucrose (2.5%, 5%, 7.5%) or caffeine (0.1%, 0.2%, 0.3%). The scale ranged from 0 to 10 units.

The influence of CMCLV on the sensory profile of polyphenolic extracts (CA, CA+CMCLV, CE and CE+CMCLV) was evaluated with the method of quantitative descriptive analysis (QDA) [Stone & Sidel, 1993; Lawless & Heymann, 1999]. Prior to the analysis, vocabularies of taste attributes for the extract were developed by the panelists using a standarized procedure [ISO/DIS 13299.2:1998]. Extracts (10 mL) in coded cups were presented to subjects in random order. The intensity of attributes was measured on a linear scale anchored "none" to "very intensive". The results were then converted to numerical values and expressed in conventional units. The attributes rated by the panel and their definitions are presented in Table 2.

All the sensory assessments were carried out at a sensory laboratory room which fulfils the requirements of the ISO standards [PN-ISO 8589: 1988]. The results are based on means from nine individual issues of three replications.

Statistical analysis

ANOVA was used to test statistical differences in chemical data as well as sensory results. Treatment means were compared using the Fisher's protected least significant difference (LSD) test. Statistical significance was considered at $p<0.05$. Statistical analysis was performed using statSoft Inc., v. 7.1 software package (Tulsa, OK, USA).

RESULTS AND DISCUSSION

In order to determine the ability of CMCs to mask the astringency a model system was used in order to eliminate the sources of interaction. The manner of *c** calculations for particular CMCs (CMCLV, CMCMV and CMCHV) is shown in Figure 1. The concentrations *c** and the concentrations above and below *c** of individual CMCs in matrices are given in Table 1. In a model system tannic acid was employed as a referential standard of astringency. This substance is a typical

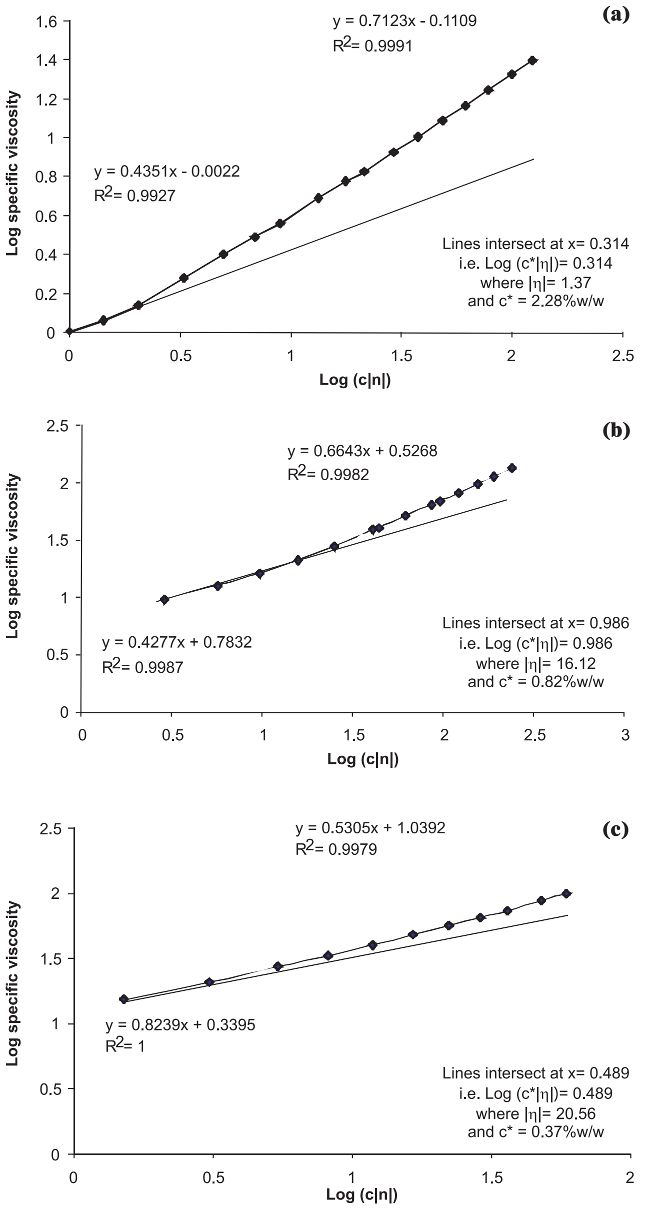

FIGURE 1. Determination of c* concentration for CMCs: (a) CMCLV; (b) CMCMV; (c) CMCHV.

TABLE 1. The critical coil overlap concentration (c*) of hydrocolloids and concentrations above and below c* used in the experiment.

CMCs	c* (%)	Concentrations above and below c* (%)
CMCLV	2.28	1.14; 3.42
CMCMV	0.82	0.41; 1.23
CMCHV	0.37	0.18; 0.55

astringent compound often used as a standard in a sensory analysis. Preliminary tests showed that 0.2% concentration of tannic acid was above the threshold level but still within the range found in plants.

Figure 2 shows the effects of CMC matrices on the astringency of tannic acid as a function of hydrocolloid concentrations. The results indicated that the ability of hydrocolloids to mask the astringency was similar and depended mostly on their concentration. It was found that the astringency in aqueous solutions was strongly reduced above the point of random coil overlap (c*) of hydrocolloids, *i.e.* at the concentration at which individual polymer chains start to overlap and which is associated with a sharp increase in the solution viscosity. The percentage of unreduced astringency above *c** values ranged from 32.5% (CMCMV) to 37.5% (CMCLV) whereas for c* these values were from 50.0% (CMCHV) to 57.5% (CMCMV). Below *c** values the percentage of unreduced astringency ranged from 72.5% (CMCHV) to 75% (CMCLV and CMCMV). On the basis of the results obtained it can be stated that all three types of the CMCs in the concentrations above *c** were the best astringency maskers. Nowadays, little is known about the mechanisms which may mask the astringency. It seems likely that the viscosity of the hydrocolloids may reduce the perceived friction when the salivary lubrication is reduced by precipitation or binding salivary proteins by astringents. We hypothesized that viscosity of CMCs was not the only factor responsible for variation in astringency since individual CMCs were observed to similarly mask astringency despite various viscosity. It suggests that the changes in the perceived astringency might have been caused by an interaction between CMCs and a phenolic compound.

The results of interactive effects of CMCLV and sucrose as well as CMCLV and caffeine in reducing astringency are presented in Figure 3a and b. It was demonstrated that astringency of 0.2% tannic acid was statistically significantly higher in sucrose solutions with CMCLV addition than it those

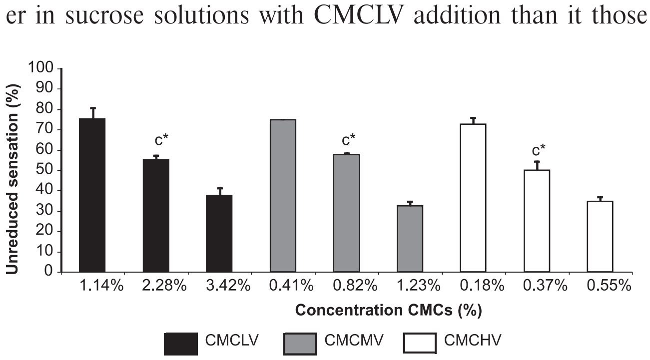

FIGURE 2. Percentage of unreduced sensation of astringency as a function of CMCs concentration.

containing CMCLV. The intensity of astringency sensation for 2.5%, 5% and 7.5% sucrose solutions without CMCLV accounted for 6.6 units, 5.2 units and 4.5 units, whereas for solutions with the addition of CMCLV for 2.7 units, 1.4 units and 0.5 units, respectively. A similar trend was observed in the caffeine. In the case of its 0.1%, 0.2% and 0.3% solutions without CMCLV the intensity of astringency reached, respectively: 1.7 units, 1.2 units and 1 unit, while in the case of its solutions with CMCLV it reached merely 0.5 units, 0.4 units and 0.2 units. On the basis of the results obtained it can be stated that the intensity of astringency could be significantly modified by altering sweetness, bitterness and viscosity.

For better recognition of the effect of CMCs on astringency of polyphenols, an extract of chokeberry fruits was applied in sensory and chemical analyses. Using the QDA method, three attributes describing taste were selected for the investigated extracts (CA; CA + CMCLV; CE and CE + CMCLV) and described in Table 2. The mean values of intensity of these attributes and the analysis of variance are presented in Table 3. Sensory profiles of extracts based on mean values were dis-

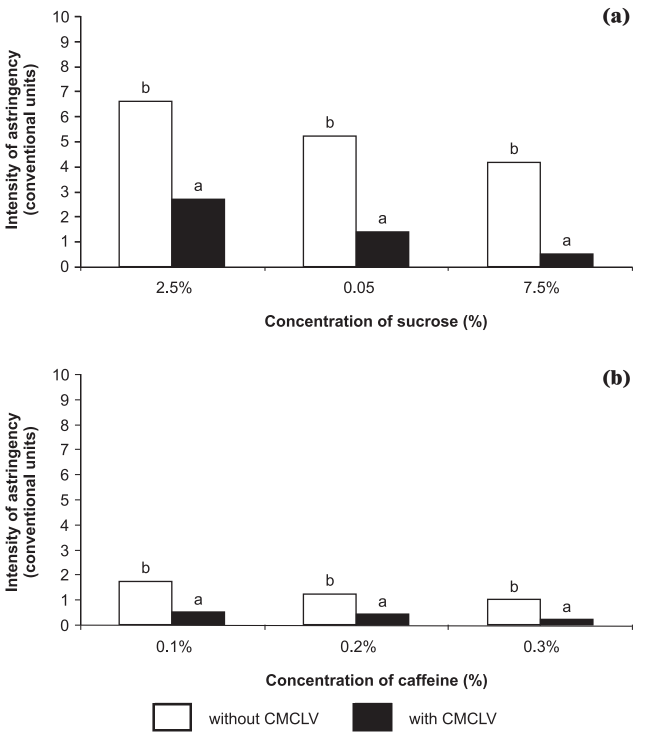

FIGURE 3. Average perceived astringency (n=9) of a 0.2% tannic acid with CMCLV and without CMCLV at different concentrations of (a) sucrose and (b) caffeine.
Means marked with the same letters are not significantly different (LSD test, $p<0.05$) for each concentration.

TABLE 2. Taste attributes definitions for the profiling of chokeberry extracts.

Attributes	Definition of the attribute
Astringent	The intensity of dryness, roughness and puckerness in the mouth (reference sample: tannic acid in water 0.2%)
Sour	Sour note characteristic for citric acid (reference sample: citric acid in water 0.15%)
Fruity	Typically associated with chokeberry fruits

TABLE 3. Intensity of sensory attributes in chokeberry extracts (scale range 0 – 10).

Extract	Sensory attributes		
	Astringent	Sour	Fruity
CA	6.2[c]	3.2[a]	3.4[a]
CA + CMCLV	3.6[a]	3.8[ab]	3.6[a]
CE	4.0[a]	4.6[b]	5.2[b]
CE + CMCLV	2.1[b]	5.9[c]	5.4[b]

Means marked in each column with the same letters are not significantly different (LSD test, $p<0.05$).

TABLE 4. Content of anthocyanins and tannins in chokeberry extracts.

Extracts	Anthocyanins (mg/g)*	Tannins	
		BSA precipitation (A_{510}/g)	HSPs precipitation (A_{510}/g)
CA	431.73±0.90[d]	106.27±1.18[d]	66.56±0.65[d]
CA + CMCLV	401.68±2.36[c]	84.38±1.32[c]	34.46±0.27[b]
CE	377.71±1.17[b]	79.90±0.53[b]	63.05±0.79[c]
CE + CMCLV	348.25±1.93[a]	39.41±0.18[a]	29.44±0.42[a]

* As cyanidin-3-5-diglucoside equivalent; values followed by the same letter in the same column are not significantly different (LSD test, $p<0.05$), letters describe comparison between all extracts.

played as spider diagrams in Figure 4a and b. At the first glance one could see that the profiles of samples were differentiated. The CA extract was distinctly more astringent yet less sour and fruity than the CE extract. It was found that the addition of CMCL caused almost a twofold decrease in the intensity of astringency of both extracts as well as an increase of their sourness. In turn, it had no statistically significant effect on the intensity of the fruity attribute typical of chokeberry fruits.

Determination of tannins in the extracts was carried out with the spectrophotometric method based on a biological property of these compounds since the astringents, such as polyphenols, have been shown to display a high affinity for binding with protein in saliva [Hagerman & Butler, 1981; Wróblewski *et al*., 2001; Bennick, 2002]. The results of precipitation capacity of proteins by phenolic extracts with and without hydrocolloid are presented in Table 4. It should be emphasized that the acetone-water system extracted more tannins than the ethanol-water system. The observed better extractivity of tannins with aqueous acetone confirms results of earlier investigations [Troszyńska *et al*. 2007b]. It was found out that the extracts were characterised by different proteins (BSA and HSPs) precipitation capacity expressed as the absorbance values at 510 nm per gram of sample. The samples containing hydrocolloid showed less efficient precipitation ability of proteins than the samples without hydrocolloid. The anthocyanins of extracts showed a similar trend as proteins precipitation capacity (Table 4). A higher content of these compounds was noted for the extracts without hydrocolloid as compared to the hydrocolloid-containing ones. The results obtained in the chemical system correspond to those described in sensory analysis (Table 3 and Figure 4) and indicate that CMCs may complex with polyphenols and reduce the efficiency of their binding with protein, and hence their astringency.

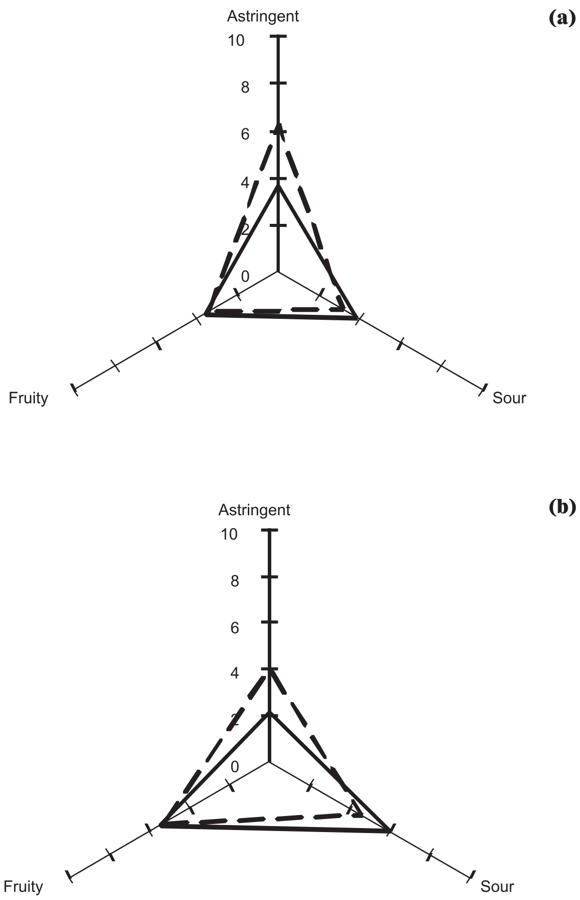

FIGURE 4. Spider diagrams of sensory profiling of chokeberry extracts: (a) CA (– – –) and CA + CMCLV (—); (b) CE (– – –) and CE + CMCLV (—);

CONCLUSIONS

In the conclusion we found that CMCs (CMCLV, CMCMV and CMCHV) above the critical concentrations (c*) were the best astringency maskers. The results indicated that the addition of CMCLV to sucrose and caffeine solutions lowered the intensity of astringency of tannic acid. The incorporation of results into the industry may be helpful in enhancing the acceptability of health-promoting beverages, for example chokeberry juices, as astringency is the major factor limiting their consumption.

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