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1           **Importance of digestive mucus and mucins for designing new**  
2                                   **functional food ingredients**

3

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13

14 **Abstract**

15           The mucus, mainly composed of the glycoproteins mucins, is a rheological substance  
16 that covers the intestinal epithelium and acts as a protective barrier against a variety of  
17 harmful molecules, microbial infection and varying lumen environment conditions.  
18 Alterations in the composition or structure of the mucus could lead to various diseases such as  
19 inflammatory bowel disease or colorectal cancer. Recent studies revealed that an exogenous  
20 intake of probiotic bacteria or other dietary components (such as bioactive peptides and  
21 probiotics) derived from food influence mucus layer properties as well as modulate gene  
22 expression and secretion of mucins. Therefore, the use of such components for designing new  
23 functional ingredients and then foods, could constitute a novel approach to preserve the  
24 properties of mucus. After presenting some aspects of the mucus and mucins in the  
25 gastrointestinal tract as well as mucus role in the gut health, this review will address role of  
26 dietary ingredients in improving mucus / mucin production and provides new suggestions for  
27 further investigations of how dietary ingredients / probiotics based functional foods can be  
28 developed to maintain or improve the gut health.

29 Keywords: intestinal mucosa; mucins; digestive tract; probiotic bacteria; food ingredients;  
30 functional foods

31

## 32        **1. Introduction**

33            The human digestive tract consists of a stable alliance among the epithelial barrier, the  
34 immune system and the resident microbiota. Besides its implication in absorption and  
35 assimilation of nutrients, exclusion of undigested food, protective role against harmful  
36 molecules and pathogenic microorganisms, the contribution of the digestive tract to sensory  
37 functions including the innate and adaptive immune responses have also been well  
38 documented. The epithelium of the gastrointestinal tract (GIT) consists of enterocytes, goblet  
39 cells, enteroendocrine cells, Paneth cells, microfold cells (M cells), cup and tuft cells, and is  
40 coated with a gel layer, called mucus (Ahluwalia, Magnusson, & Lohman, 2017; McGuckin,  
41 Thornton, & Whitsett, 2015). Furthermore, the human GIT also harbors more than  $10^{13}$  of  
42 resident bacteria per gram of feces colonizing the mucus layer (Sender, Fuchs, & Milo, 2016).  
43 The number of microbial cells as well as the number of species varies throughout the GIT (Li  
44 et al., 2015) with few resident bacteria in the stomach and small intestine (Bik et al., 2006),  
45 and a dramatic increase in the distal ileum and the colon (Ermund, Schütte, Johansson,  
46 Gustafsson, & Hansson, 2013; Li et al., 2015). The association of bacteria with the host have  
47 led to co-evolution with mutual benefits and impacts (Bäckhed, Ley, Sonnenburg, Peterson, &  
48 Gordon, 2005; Liang, Leung, Guan, & Au, 2018). Hence, the resident bacteria could influence  
49 the composition and structuration of the mucus layer and finally the mucus properties  
50 (Jakobsson et al., 2015).

51            The digestive mucus, mostly consisting of mucins, plays a major role in the good functioning  
52 of the digestive tract and thereby the human health. Whatever the reasons, alterations in  
53 composition or structure of the mucus result in a wide variety of diseases including  
54 inflammatory bowel diseases or colorectal cancer (Johansson, Sjövall, & Hansson, 2013;  
55 Velcich et al., 2002). Hence, mucins, such as MUC2 (“MUC” refers to human mucins

56 whereas “Muc” corresponds to mucins in mice and other animals), constitute a target for the  
57 design of new therapeutic molecules because of their involvement in tumorigenesis (Aithal et  
58 al., 2018; Li et al., 2018).

59 Scientific community and public are now more interested in natural products to maintain  
60 consumer health. This is reflected by the ever-growing market of functional food products  
61 containing health-promoting ingredients and / or bacteria (Douillard, & de Vos, 2019;  
62 Vicentini, Liberatore, & Mastrocola, 2016). Therefore, it would be interesting to explore the  
63 possibility of identifying functional ingredients able to modulate mucus production as the  
64 drug treatments are usually associated with adverse effects. After a brief description of the  
65 mucus, its beneficial role in maintaining human health and the control of this layer, this  
66 review will address the effects of food ingredients on mucus or mucins production and the  
67 possible ways to design / improve functional ingredients, a first step in developing functional  
68 foods, summarizing thereby the recent insights in this domain.

69

## 70 **2. Overview of digestive mucus and its beneficial role**

71 Mucus, secreted by intestinal goblets cells, is characterized by its viscosity and  
72 rheological properties. It consists in water ( $\approx 95\%$ ), electrolytes, enzymes, salts, DNA, lipids,  
73 growth factors, antibacterial peptides and proteins such as defensins, immunoglobulins,  
74 lysozymes and trefoil factors (McGuckin, Thornton, & Whitsett, 2015), and especially  
75 glycoproteins called mucins. It not only constitutes a lubricant facilitating the transport of the  
76 chyme along the GIT (Corfield, 2015; Lai, Wang, Wirtz, & Hanes, 2009) but also serves as a  
77 barrier that protects the epithelial mucosa against mechanical injuries as well as from harmful  
78 enteric pathogenic bacteria, viruses and parasites (Birchenough, Johansson, Gustafsson,

79 Bergström, & Hansson, 2015; Ermund, Schütte, Johansson, Gustafsson, & Hansson, 2013;  
80 Liévin-Le Moal & Servin, 2006; Pelaseyed et al., 2014). Moreover, it also plays a key role in  
81 maintaining intestinal homeostasis (Faderl, Noti, Corazza, & Mueller, 2015).

82 Studies conducted in rodent models and humans have shown that the mucus organization and  
83 composition differ along the GIT (Figure 1). In the small intestine, there is a single,  
84 discontinuous and disorganized mucus layer which is continuously secreted from the crypt  
85 openings (Atuma, Strugala, Allen, & Holm, 2001; Ermund, Schütte, Johansson, Gustafsson,  
86 & Hansson, 2013; Pelaseyed et al., 2014). In the stomach and the colon, the mucus is thicker  
87 and composed of two layers. The inner dense layer is strongly attached to the epithelium,  
88 devoid of bacteria and is constantly renewed every 1-2 hours by surface goblet cells of the  
89 colon (Ermund, Schütte, Johansson, Gustafsson, & Hansson, 2013; Gustafsson et al., 2012;  
90 Johansson, 2012). The outer loose layer, facing the lumen, is disorganized, more hydrated and  
91 thicker than the inner layer and is colonized by the commensal bacteria in the colon (Atuma,  
92 Strugala, Allen, & Holm, 2001; Ermund, Schütte, Johansson, Gustafsson, & Hansson, 2013;  
93 Hansson & Johansson, 2010; Johansson et al., 2008). It results from the inner layer probably  
94 through the proteolytic cleavage occurring within the mucins (Johansson et al., 2008).

95         As major constituents of mucus, mucins represent large and highly glycosylated  
96 proteins expressed in different organs. In the adult human digestive tract, a wide variety of  
97 mucins are secreted at the epithelium surfaces (Figure 1). They are clustered into three distinct  
98 subfamilies: i) secreted gel-forming mucins, ii) secreted non-gel forming mucins, iii)  
99 membrane-bound mucins, and their physiological roles are described in Table 1 (Moniaux,  
100 Escande, Porchet, Aubert, & Batra, 2001). Being highly glycosylated molecules, a high  
101 proportion of mucin's molecular weight corresponds to carbohydrates (oligosaccharides or

102 glycan chains). Their structure described in Figure 2 is important for both viscoelasticity  
103 (Demouveau, Gouyer, Gottrand, Narita, & Desseyn, 2018) and bioactivity of mucus (Petrou,  
104 & Crouzier, 2018). Biosynthesis and secretion of all types of mucins present some similarities  
105 such as N-glycosylation in the endoplasmic reticulum and O-glycosylation at PTS sequence  
106 (proline, threonine and serine rich region) in the Golgi apparatus. Afterwards, membrane  
107 bound mucins are inserted into the membrane *via* C-terminal transmembrane domain whereas  
108 secreted mucins are oligomerized and packed into Goblet cell granules for secretion (Figure  
109 3).

110 Mucins play a major role in the protection and functioning of different parts of the GIT. At  
111 the oral sphere level, mucins of saliva, e.g. MUC5B and MUC7 are involved in maintenance  
112 of its rheological properties and protect the floor of the mouth against mechanical forces  
113 during eating and speaking. They also protect from different diseases by reducing bacterial  
114 attachment and / or their biofilm formation capacity (Table 1 and Figure 1) (Amerongen,  
115 Bolscher, & Veerman, 1995; Dawes et al., 2015; Plummer & Douglas, 2006; Stinson et al.,  
116 1982; van't Hof, Veerman, Nieuw Amerongen, & Ligtenberg, 2014). Other mucins of oral  
117 sphere (MUC1, MUC16 and MUC4) that line the duct of parotid, submandibular and minor  
118 salivary glands are involved in cell signal transduction and binding of secreted mucins  
119 MUC5B and MUC7 (Frenkel & Ribbeck, 2015b; Liu et al., 2002; O'Brien, Beard,  
120 Underwood, & Shigemasa, 2002; Sengupta et al., 2001). In the rest of the GIT, the mucins  
121 safeguard the cell surface epithelium from the endogenous toxic molecules and pathogenic  
122 bacteria (Table 1 and Figure 1) (Allen & Flemström, 2005; Bevins, 2006). Consequently, they  
123 prevent from different diseases such as ulcerative colitis and colon cancer (Johansson et al.,  
124 2008; Sluis et al., 2006; Velcich et al., 2002). In addition, the alteration of mucus  
125 glycosylation pattern impacts both the composition of intestinal microbiota and the gut

126 architecture in mouse models (Arike & Hansson, 2016; Sommer et al., 2014; Staubach et al.,  
127 2012). For example, it has been demonstrated that the loss of mucin glycosylation especially  
128 the Core 1–derived *O*-glycans (the predominant form of mucin *O*-glycans in mice) led to the  
129 development of spontaneous colitis in a mouse model (Fu et al., 2011).

130

### 131 **3. Control of mucus layer, mucin synthesis and associated diseases**

132 Under normal physiological conditions, mucus is regularly renewed and this phenomenon  
133 results from a balance between mucus degradation and synthesis. Intestinal bacteria take  
134 important part in regenerating the mucus layer. The establishment of mucus-degrading  
135 bacteria in the host intestine starts few months after birth and is completed gradually with age  
136 (Palmer, Bik, DiGiulio, Relman, & Brown, 2007). This process is not only linked with the  
137 individual age but also with the diet. Midtvedt et al. (1994) have observed that the  
138 establishment of mucin degradation bacteria is delayed at least 4 months in breast-fed  
139 children than those fed with formula milk (Midtvedt, Carlstedt-Duke, & Midtvedt, 1994).  
140 Among intestinal bacteria known to have a mucolytic activity, *Akkermansia muciniphila* is  
141 one of the bacteria having large enzymatic machinery for mucins oligosaccharides breakdown  
142 (Derrien, Vaughan, Plugge, & de Vos, 2004; Derrien, Collado, Ben-Amor, Salminen, & de  
143 Vos, 2008). In mouse, it is one of the first users of mucus (Berry et al., 2013). This bacterium  
144 hydrolyzes mucins oligosaccharides and releases sugar residues such as N-acetylglucosamine,  
145 N-acetylgalactosamine, fucose or sialic acid, which can be used by other bacteria such as  
146 *Bifidobacterium infantis* (Derrien, Belzer, & de Vos, 2017; Garrido, Kim, German, Raybould,  
147 & Mills, 2011) or *Bacteroides thetaiotaomicron* (For comprehensive review see Koropatkin,  
148 Cameron, & Martens, 2012). Some other intestinal bacteria such as *B. bifidum*, *Ba. fragilis*



149 and *Ruminococcus gnavus* also present a mucolytic activity (Katoh et al., 2017; Macfarlane &  
150 Gibson, 1991; Png et al., 2010).

151 The bacteria and / or their metabolites could modulate the expression and / or the secretion of  
152 mucins (Liévin-Le Moal et al., 2002; Smirnova, Guo, Birchall, & Pearson, 2003), the  
153 development of the mucus secreting goblet cells as well as the mucin glycosylation profile. It  
154 has been shown that *Ba. thetaiotaomicron* influenced the development of goblet cells in  
155 mono-associated rats by stimulating the expression of *klf4* gene encoding a transcription  
156 factor involved in goblet cell differentiation. This study also reported that this bacterium  
157 induced the expression of some genes encoding mucins (*muc2* and *muc4*) in the gnotobiotic  
158 rats compared to germ free model. Furthermore, the presence of two metabolically  
159 complementary bacteria, *Ba. thetaiotaomicron* and *Faecalibacterium prausnitzii* in  
160 gnotobiotic rats modulates the mucin glycosylation and goblet cells development in the  
161 colonic epithelium compared to germ free rats (Wrzosek et al., 2013).

162 Abnormal synthesis and / or processing of mucins leads to disruption of mucus structure and  
163 imbalance of mucosal barrier function. This could favour the development of a pathogen  
164 bacterium which becomes able to circumvent the protective function of the mucus barrier  
165 (Navabi, Johansson, Raghavan, & Lindén, 2013). Likewise, the abnormal functioning of the  
166 mucosal barrier leads to the development of intestinal diseases such as Inflammatory Bowel  
167 Disease (Ulcerative colitis, Crohn's disease) and eventually colorectal cancer (Bergstrom et  
168 al., 2017; Velcich et al., 2002; Wenzel et al., 2014). Patients with ulcerative colitis have  
169 shown modifications in mucus layer properties, mucin gene expression, mucin proteins  
170 synthesis as well as mucin's glycosylation structure (O-glycosylation, sulfation, and  
171 silylation) leading thus to a deficient mucus barrier (Bergstrom et al., 2017; Yamamoto-

172 Furusho, Ascaño-Gutiérrez, Furuzawa-Carballeda, & Fonseca-Camarillo, 2015). For instance,  
173 it has been reported that patients with active ulcerative colitis showed a significant decrease in  
174 gene and protein expression and production of MUC20 in epithelial cells from mucosa as well  
175 as submucosa (Yamamoto-Furusho, Ascaño-Gutiérrez, Furuzawa-Carballeda, & Fonseca-  
176 Camarillo, 2015). In addition, in mice model lacking core 2  $\beta$ 1,6-N-acetylglucosaminyl-  
177 transferase enzyme which initiates core 2 type protein O-glycosylation in mucin protein, an  
178 increase in intestinal permeability and susceptibility to experimental colitis (Dextran sulfate  
179 sodium induced colitis) has been observed (Stone et al., 2009). Furthermore, in Muc2-  
180 deficient mice, the bacterial contact with the epithelium explains the inflammation and the  
181 development of the cancer in these animals (Johansson et al., 2008).

182

#### 183 **4. Impact of food components on mucus and mucins**

184 As discussed before, the control of mucus layer plays an essential role in maintaining health  
185 and should constitute a major target for conception of preventive strategies, including specific  
186 diets. Although knowledge about the impact of food ingredients on the digestive mucus layer  
187 remain relatively scarce. However, different dietary components such as milk proteins,  
188 bacteria or their metabolites, bioactive molecules (released either by bacteria in certain  
189 fermented foods or derived directly from food after digestion), fiber and short chain fatty  
190 acids have been shown to modulate the mucin gene expression and secretion *via* interaction  
191 with intestinal mucosa (Mack, Michail, Wei, McDougall, & Hollingsworth, 1999; Martinez-  
192 Maqueda, Miralles, Cruz-Huerta, & Recio, 2013; Rokana, Singh, Mallappa, Batish, & Grover,  
193 2016; Burger-van Paassen et al., 2009). Hence, the positive effects of different food

194 components on intestinal mucus properties particularly mucin modulation are discussed  
195 hereafter.

196

#### 197 **4.1. Effect of lactic acid and probiotic bacteria on mucin gene expression and** 198 **secretion**

199 Lactic Acid Bacteria (LAB) or probiotic bacteria present in fermented milk products can  
200 modify mucus layer by regulating mucins expression. Such modulation has been shown to  
201 occur either by direct contact of bacteria with mucosal surfaces or by bacterial products  
202 (Liévin-Le Moal et al., 2002; Raja, Murali, Devaraj, & Devaraj, 2012; Smirnova, Guo,  
203 Birchall, & Pearson, 2003). It has been demonstrated that *Lactobacillus. plantarum* 299v and  
204 *Lb. rhamnosus* GG significantly increased the mucosal layer and mucosal integrity through  
205 upregulated expression of MUC3 which inhibited adherence of the enterohaemorrhagic  
206 *Escherichia coli* O157:H7 to intestinal epithelial cells HT29 (Mack, Ahrne, Hyde, Wei, &  
207 Hollingsworth, 2003). In the same perspective, Matter and coworkers have also reported that  
208 the expression of *MUC2* gene was induced at transcriptional and translational levels in Caco-  
209 2 cell cultures by *Lb. rhamnosus* GG (Mattar et al., 2002). Similarly, intestinal integrity was  
210 improved when mice were treated with *Lb. rhamnosus* CNCM I-3690. This was mainly due to  
211 enhanced production of goblet cells and consequently increased expression of mucin MUC2  
212 (Martín et al., 2019). The treatment of Caco-2 cells with a cow milk, initially fermented with  
213 *Lb. paracasei* CBAL74 and heated to kill bacteria, led to increase of *MUC2* gene expression  
214 and mucus layer thickness compared to non fermented milk (Paparo et al., 2018). Thus, this  
215 study highlighted that the increased mucus production is not restricted to living probiotic  
216 bacteria but their components could also preserve the mucus layer integrity. Similarly, it has  
217 been shown that administration of milk fermented with *Lb. plantarum* MTCC 5690 enhanced

218 the intestinal barrier function by increasing expression of MUC2 in salmonella-infected mice  
219 compared to those fed with unfermented probiotic milk (Rokana, Singh, Mallappa, Batish, &  
220 Grover, 2016). Besides the use of individual probiotic strains or probiotic-fermented milk, the  
221 oral administration of VSL#3 probiotic mixture (containing different species of  
222 *Lactobacillus*, *Bifidobacterium*, and one species of *Streptococcus*) for seven days enhanced  
223 by 60 % basal luminal mucin content in rats. In addition, VSL#3 directly inoculated into  
224 colonic loops significantly enhanced Muc2 mRNA expression compared to Muc1 and Muc3  
225 for which only a slight increase was observed (Caballero-Franco, Keller, De Simone, &  
226 Chadee, 2007). Surprisingly, these results obtained *in vivo* were not reproducible *in vitro*  
227 when authors incubated VSL#3 bacterial cultures with cultured LS 174T intestinal epithelial  
228 cells suggesting that the cell culture conditions were not optimal for proper growth of  
229 probiotic bacteria. On the other hand, the authors explored the fact that bacterial metabolites  
230 could induce the mucin secretion in LS 174T cells. They reported that the bacterial secreted  
231 products from VSL#3 contained in the conditioned media remarkably stimulated mucin  
232 secretion and the stimulatory effect was similar to that obtained from the *in vivo* experiments  
233 (Caballero-Franco, Keller, De Simone, & Chadee, 2007). More recently, this probiotic  
234 mixture has been shown to reduce colonic inflammation and improve intestinal barrier  
235 function in Muc2 mucin-deficient mice indicating that VSL#3 did not require an intact mucin  
236 barrier to exert its beneficial effects (Kumar, Kisson-Singh, Coria, Moreau, & Chadee,  
237 2016). Finally, it has been shown that despite poor mucus-adhesion, *S. thermophilus* promotes  
238 mucus pathway in digestive tract of gnotobiotic rats by producing lactate which in turn  
239 increased the number of goblet cells and amount of mucus inducer KLF4 transcription factor.  
240 A significant increase in KLF4 protein due to lactate production by this bacterium was also  
241 reported in HT29-MTX cells (Fernandez et al., 2018).

242

#### 243 **4. 2. Impact of bioactive peptides on mucin expression and secretion**

244 In addition to bacteria, hydrolysates from milk proteins have been shown to affect mucin  
245 expression and secretion in the intestinal mucosa (Table 2). Indeed, these proteins harbor  
246 bioactive sequences released either after digestion (*in vivo* or *in vitro*) or under the effect of  
247 proteolytic system of bacteria such as LAB during processing of fermented milks. These  
248 proteins provide peptides with different positive health effects on the host, including blood  
249 pressure lowering, immunomodulatory, antimicrobial, cholesterol-lowering and antioxidant  
250 effects. Several studies performed either *in vitro* or *in vivo* have also reported their impacts on  
251 the modulation of mucin secretion (Claustre et al., 2002; Han, Deglaire, Sengupta, &  
252 Moughan, 2008 ; Plaisancié et al., 2013; Plaisancié et al., 2015; Trompette et al., 2003). It has  
253 been shown that the luminal administration of casein and lactalbumin hydrolysates separately  
254 in the isolated perfused rat jejunum induced release of mucins. Conversely, the administration  
255 of native caseins did not significantly stimulate mucin secretion (Claustre et al., 2002).  
256 Likewise, when rats were fed for 8 days with test diets enriched either with hydrolyzed casein  
257 or a synthetic amino acid mixture as well as a protein-free diet, hydrolyzed casein enriched  
258 diet significantly up-regulated the expression of Muc3 and Muc4 mRNA in the small  
259 intestinal tissue and the colon, respectively, compared to other diets (Han, Deglaire, Sengupta,  
260 & Moughan, 2008). Fernández-Tomé and colleagues also reported significantly higher fecal  
261 mucin (*via* O-linked glycoproteins dosage in feces) content after 2- and 8-weeks  
262 administration of casein hydrolysate in rats. Indeed, long term intake of casein hydrolysate  
263 resulted in an increased expression of Muc2 and Muc3 mRNA in the ileum and the colon of  
264 rats (Fernández-Tomé et al., 2017). Furthermore, mucin secretion was escalated when  $\beta$ -  
265 casomorphin-7, an opioid peptide from bovine  $\beta$ -casein, was administered in the lumen of the

266 isolated vascularly perfused rat jejunum (Trompette et al., 2003). Further studies conducted  
267 with  $\beta$ -casomorphin-7 in intestinal mucin-producing cells of rat (DHE) and human (HT29-  
268 MTX) revealed an increased expression of different mucins in a time and a dose dependent  
269 manner. In DHE cells, Muc2 and Muc3 expression was enhanced whereas in case of HT29-  
270 MTX, an increase of transcription as well as secretion of MUC5AC was reported. The authors  
271 concluded that this peptide contributed significantly to mucin secretion *via* a direct effect on  
272 intestinal goblet cells and the activation of  $\mu$ -opioid receptors (Zoghbi et al., 2006). Other  
273 studies, performed with the human HT29-MTX cells, have also reported the modulation of  
274 mucin gene expression and stimulation by the total peptide pool derived from a fermented  
275 milk (yogurt) (Plaisancié et al., 2013; Plaisancié et al., 2015). Indeed, these peptides  
276 stimulated *MUC2* and *MUC4* gene expression as well as mucin secretion in HT29-MTX cells  
277 (Plaisancié et al., 2013). Interestingly, this study has identified peptide sequences carrying  
278 biological activity for further tests in rat models. Thereby, the oral administration of the  
279 peptide fraction (f94-123) of  $\beta$ -casein to pups enhanced the number of goblet cells and Paneth  
280 cells along the small intestine which were associated with higher expression of intestinal  
281 mucins (Muc2 and Muc4) and antibacterial factors (Plaisancié et al., 2013). Furthermore,  
282 from the  $\beta$ -casein peptide sequence (f94-123), shorter peptides having different biological  
283 activities were also identified. These peptides up-regulated MUC4 expression in HT29-MTX  
284 cells whereas no effect was observed on the levels of secreted mucins MUC2 and MUC5AC.  
285 In contrast, other identified shorter peptide sequences increased the gene expression of *MUC2*  
286 and *MUC5AC* mucins (Plaisancié et al., 2015). In another study conducted in HT29-MTX  
287 cells, the stimulatory effect of casein hydrolysate and isolated peptides on mucin production  
288 was also investigated. Casein hydrolysate and casein derived peptides mainly  $\alpha_{S1}$ -casein  
289 (f143-149) (AYFYPEL) and  $\alpha_{S1}$ -casein (f144-149) (YFYPEL) significantly enhanced the

290 expression of mucin gene encoding MUC5AC (over 1.7-fold basal level) after 4 h of  
291 exposure. The authors reported that casein hydrolysate containing both above cited peptides  
292 displayed a higher effect on mucin secretion as determined from mucin-like glycoproteins  
293 quantification than individual peptides (Martinez-Maqueda, Miralles, Cruz-Huerta, & Recio,  
294 2013). Moreover, whey-derived peptides,  $\alpha$ -lactorphin and  $\beta$ -lactorphin with described or  
295 probable opioid activity were also shown to induce secretion of MUC5AC mucin in HT29-  
296 MTX cells. Concerning the gene expression level of this mucin, it was up-regulated by  
297  $\alpha$ -lactorphin in these cells (Martinez-Maqueda et al., 2012). All these findings indicate that  
298 peptides derived from yogurt may play an important role in protecting intestinal lumen from  
299 damaging agents by modulating Paneth and mucus cells, which are known to play a crucial  
300 role in intestinal protection (Plaisancié et al., 2013). Hence, the presence of bioactive peptides  
301 including those probably released from LAB in dairy products from milk proteins could  
302 thereby improve gut health in the host.

### 303 **4.3. Impact of other food ingredients on mucin expression and secretion**

304 Besides milk proteins, rye bran, oat bran and soybean hull (Table 2) were also shown  
305 to increase goblet cell volume density in the proximal and distal small intestine of hamsters  
306 (Lundin et al., 1993). Food fibers can also positively impact the mucus layer through the  
307 production of SCFAs, such as acetate, propionate and butyrate products, which are the  
308 metabolic products of the intestinal microbiota. Among SCFAs, butyrate appears to be the  
309 most effective in stimulating mucus secretion in rat colon (Shimotoyodome, Meguro, Hase,  
310 Tokimitsu, & Sakata, 2000). Moreover, the effect of propionate and butyrate on mucin gene  
311 expression in human goblet cell-like LS 174T cells was also demonstrated. Butyrate at 1 mM,  
312 as well as propionate induced an increase in MUC2 mRNA levels. This study has also  
313 investigated the mechanisms that regulate butyrate-mediated effect on MUC2 synthesis. The

314 authors have revealed an active butyrate-responsive region within the MUC2 promoter and  
315 have demonstrated that the regulation of MUC2 by butyrate is associated with increased  
316 acetylation of histone H3 and H4 and methylation of H3 at the MUC2 promoter (Burger-van  
317 Paassen et al., 2009). Similar results, i.e., stimulation of MUC2 mucin and increase in histone  
318 3 acetylation were observed after treatment of LS 174T cells with 1-2 mM sodium butyrate.  
319 The secreted mucin was detected by histological PAS staining of carbohydrate chains of  
320 mucin and expression at the protein and mRNA levels was confirmed by immunoblotting  
321 with anti-MUC2 antibody and real-time RT-PCR, respectively (Hatayama, Iwashita,  
322 Kuwajima, & Abe, 2007). More recently, the variation of four mucin gene expression  
323 (*MUC2*, *MUC3*, *MUC13*, and *MUC17*) in coculture of Caco-2/HT29-MTX cells was analyzed  
324 by real-time RT-PCR in the presence of antioxidant compounds like chlorogenic acid,  
325 epicatechin gallate, and quercetin (apple, tea, and coffee polyphenols, respectively). In  
326 addition, the secretion level of MUC2 was also determined by enzyme-linked immunoassay  
327 (ELISA) analysis. After exposing the cells to sub-lethal concentrations (10  $\mu$ M) of each of the  
328 different polyphenolic compounds over a period of 48 h, it is reported that each phenolic  
329 compound induces differential expression patterns of mucin genes and only a significant  
330 increase in *MUC17* gene expression was observed especially with epicatechin gallate and  
331 quercetin. However, results from ELISA analyses showed no significant differences in  
332 secretion level of mucin MUC2 (Volstatova et al., 2019). In contrast, quercetin induced the  
333 secretion of MUC2 and MUC5AC mucins in quercetin-treated LS 174T cells. Both mucins  
334 were also detected at mRNA level. The secretion of these mucins in this cell line was  
335 attributed to enhanced extracellular signal regulated kinase (ERK)1-2 activity as its inhibition  
336 by the MEK inhibitor PD98059 reverted the stimulatory effects of quercetin. Besides LS  
337 174T cells, quercetin treatment also enhanced the mRNA levels of MUC2 and MUC5AC *via*  
338 ERK1-2 pathway in the human intestinal epithelial Caco-2 cells (Damiano et al., 2018).



339 **5. Research approaches to improve/design new functional ingredients/foods**

340 The increasing costs of health as well as the desire of numerous consumers to support a  
341 preventive rather than a curative approach have created favourable conditions to develop  
342 functional foods, which are claimed to display health specific properties because of the  
343 presence of some specific functional and / or absence of some harmful components (Gupta &  
344 Abu-Ghannam, 2012). Although the informations presented above remain scarce, the  
345 evidence provided through the action of probiotics, bioactive peptides or some polyphenolic  
346 compounds suggest that they may be valuable ingredients / tools for strengthening the  
347 digestive mucus layer or mucus barrier that is central to consumer health. However, it must be  
348 underlined that most of the work presented in this review focused on effects of tested  
349 ingredients on mucins transcription (Table 2). Nevertheless, other parameters can also be  
350 explored such as effects of food ingredients on i) the microbiota composition and equilibrium,  
351 ii) the mucus producing cells and thus on the intestinal epithelium and / or iii) glycosylation  
352 profile of mucins. The search of such ingredients constitutes a first step in designing  
353 functional foods, i.e., before including them in a food matrix, study their bioavailability in this  
354 matrix during digestion followed by their interactions with other constituents of the diet.

355 Two types of approaches can be used to identify functional ingredients directed towards  
356 mucus and mucins. The first is to screen high throughput probiotic strains, bioactive peptides  
357 or other dietary compounds for their adhesion capacity (for probiotics) and / or their ability to  
358 induce the production of mucins (induction of transcription/translation/secretion) using cell  
359 models. Indeed, several cell lines are known to exhibit the functional characteristics of  
360 intestinal cells found in small and large intestines and express different mucins (Table 3).  
361 Such screening can concern new probiotic strains or food ingredients, but also ingredients  
362 already described for other properties such as the opioid bioactive peptides (see above). The

363 second approach consists of using animal models to explore the capacity of some ingredients  
364 to increase the goblet cells and then the mucus production or modulate the microbiota and the  
365 glycosylation profile of mucins, a path that seems insufficiently explored so far. This implies to  
366 be able to characterize the microbiota rapidly and on cohorts, to determine which type of flore  
367 is favored, how does it is maintained and what are the determinants. In addition; it could be  
368 interesting to explore the relationship between food ingredients and the glycosylation state of  
369 mucins.

370 To conclude, given the impact of diseases related to dysfunction of the digestive tract and  
371 especially the amount of mucus and mucins produced, it is necessary to intensify research in  
372 the field of functional ingredients with the prospect of exploiting them in the maintenance of  
373 gut health.

374

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380       **6. References**

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795

**Table 1** Mucins in the digestive tract, their localization, cell expression and physiological functions

796

Mucins	Expression in each digestive tract segment	Cell type expression (Johansson, Sjövall, & Hansson., 2013)	Function	References
<b>Secreted mucins</b>				
<b>- Gel-forming mucins</b>				
<b>MUC2</b>	Small intestine (jejunum and ileum), colon	Goblet cells, Paneth cells	Formation of a protective barrier, lubrication, entrapment	Bäckström, Ambort, Thomsson, Johansson, Sjövall & Hansson, 2013 Nilsson et al., 2014 Kawashima, 2012
<b>MUC5AC</b>	Stomach	Mucous cells	Formation of a protective barrier, lubrication, entrapment	Allen & Flemström, 2005 Ho et al., 2004
<b>MUC5B</b>	Salivary glands, submandibular glands (mouth)	Mucous cells, Goblet cells	Lubrication, pathogens entrapment	Al-Hashimi & Levine, 1989 Frenkel & Ribbeck, 2015a, 2015b
<b>MUC6</b>	Stomach, ileum	Mucous cells	Protection, lubrication, entrapment	Toribara et al., 1993 Ho et al., 2004
<b>- Non gel-forming mucins</b>				
<b>MUC7</b>	Salivary glands (mouth)	Mucous cells	Clearance of harmful substances and pathogenic bacteria	Bobek, Tsai, Biesbrock, & Levine, 1993; Frenkel & Ribbeck, 2015b; Mehrotra, Thornton, & Sheehan, 1998; Plummer & Douglas, 2006; Stinson et al., 1982
<b>Cell-membrane bound mucins</b>				
<b>MUC1</b>	All intestinal segments	Epithelial cells	Cellular signal transduction, adhesion properties to bacteria, barrier activity	Albrecht & Carraway, 2011 Bafna, Kaur, & Batra, 2010
<b>MUC3A/B</b>	Small intestine, colon	Enterocytes	Adhesion modulation and cell signaling	Bafna, Kaur, & Batra, 2010 Desseyn, Tetaert, & Gouyer, 2008
<b>MUC4</b>	Salivary glands, small intestine, colon	Epithelial cells, Goblet cells	Lubrication, epithelial cell differentiation	Albrecht & Carraway, 2011 Moniaux et al., 1999 Rong et al., 2005
<b>MUC12</b>	Colon	Enterocytes	Epithelial cell regulation	Desseyn, Tetaert, & Gouyer, 2008 Williams et al., 1999
<b>MUC13</b>	Small intestine, colon	Enterocytes	Epithelial barrier function	Maher, Gupta, Nagata, Jaggi, & Chauhan, 2011 Sheng et al., 2011 Williams et al., 2001
<b>MUC16</b>	Mouth	Epithelial cells	Apical surface protection	Desseyn Tetaert, & Gouyer, 2008 O'Brien, Beard, Underwood, & Shigemasa, 2002
<b>MUC17</b>	Stomach, duodenum, colon	Enterocytes	Extracellular matrix constituent, apical surface protection	Desseyn, Tetaert, & Gouyer, 2008 Gum, Crawley, Hicks, Szymkowski, & Kim, 2002



<b>Food ingredients</b>	<b>Enzyme hydrolyzed /</b>	<b>Increased mucin expression / secretion</b>	<b>Study model</b>	<b>References</b>
Casein hydrolysate	Pancreatin	Muc3, Muc4 mRNA	Male rats	Han, Deglaire, Sengupta, & Moughan 2008
Casein hydrolysate	Pepsin	Muc2, Muc3 mRNA	Male rats	Fernández-Tomé et al., 2017
$\beta$ -casomorphin-7	Purified / synthesized	Mucins	Male rats	Trompette et al., 2003
$\beta$ -casomorphin-7	Purified / synthesized	Muc2, Muc3 mRNA	Rat DHE cells	Zoghbi et al., 2006
$\beta$ -casomorphin-7	Purified / synthesized	MUC5AC	Human HT29-MTX cells	Zoghbi et al., 2006
Fermented milk (Yogurt)	Commercial product	MUC2, MUC4	Human HT29-MTX cells	Plaisancié et al., 2013
$\beta$ -casein (f94-123)	Chemically synthesized	MUC2, MUC4	Human HT29-MTX cells	Plaisancié et al., 2013
$\beta$ -casein (f94-123)	Chemically synthesized	Muc2, Muc4 mRNA	Rat pups	Plaisancié et al., 2013
$\beta$ -casein (f108-113), $\beta$ -casein (f114-119)	Purified	MUC4	Human HT29-MTX cells	Plaisancié et al., 2015
$\beta$ -casein (f94-108)	Purified	MUC2, MUC5AC mRNA	Human HT29-MTX cells	Plaisancié et al., 2015
Casein hydrolysate	Pepsin	MUC5AC mRNA	Human HT29-MTX cells	Martínez-Maqueda, Miralles, Cruz-Huerta, & Recio, 2013
$\alpha_{s1}$ -casein (f143-149) $\alpha_{s1}$ -casein (f144-149)	Synthesized	MUC5AC mRNA	Human HT29-MTX cells	Martínez-Maqueda, Miralles, Cruz-Huerta, & Recio, 2013
$\alpha$ -lactorphin	Synthesized	MUC5AC	Human HT29-MTX cells	Martinez-Maqueda et al., 2012
Sodium butyrate	Commercial	MUC2 mRNA	Human LS 174T	Burger-Van Paassen et al., 2009; Hatayama, Iwashita, Kuwajima, & Abe., 2007
Quercetin, Epicatechin	Commercial	MUC17 mRNA	Caco-2/HT29-MTX cells co-culture	Volstatova et al., 2019
Quercetin	Commercial	MUC2, MUC5AC	Human LS 174T, Human Caco-2	Damiano et al., 2018

799 **Table 3** Summary of most cell lines *in vitro* models used in bacterial adhesion studies

Cell lines	Origin and characteristics	<i>MUC</i> gene expressed	References
<b>Caco-2 TC7</b>	Human colorectal adenocarcinoma cell line which differentiate spontaneously <i>in vitro</i> forming a polarized cell monolayer. The cell monolayer is devoid of mucus	<i>MUC1, MUC3, MUC4, MUC2, MUC5AC, MUC6</i> and <i>MUC13</i>	Bernet-Camard, Coconnier, Hudault, & Servin, 1996; Bu, Li, Tian, & Huang, 2011; Fogh, Fogh, & Orfeo, 1977; Götcke et al., 1998; van Klinken et al., 1996
<b>HT-29</b>	Human colorectal adenocarcinoma cell line forming polarized cell monolayer in culture (enterocytes + goblet cells)	<i>MUC1, MUC2, MUC3</i>	Augeron & Laboisse, 1984; Dahiya et al., 1992
<b>HT29-MTX</b>	Human colorectal adenocarcinoma cell line deriving from HT-29 cell line after growth adaptation to methotrexate thus acquiring the goblet cell-like phenotype with a high capacity of mucus production	<i>MUC1, MUC2, MUC3, MUC5AC</i>	Huet et al., 1995; Lesuffleur, Barbat, Dussaulx, & Zweibaum, 1990; Lesuffleur et al., 1991; Lesuffleur et al., 1993; Lesuffleur et al., 1995
<b>HT29-Cl.16E</b>	Human colorectal adenocarcinoma cell line deriving from HT-29 cell line after growth adaptation to sodium butyrate thus acquiring the goblet cell-like phenotype with a high capacity of mucus production	<i>MUC3, MUC2</i>	Augeron & Laboisse, 1984; Jarry et al., 1994; Maoret et al., 1989

800

801

Mucus layer

Mucins

No mucus layer

Oral cavity

*MUC7, MUC5B,*  
MUC1, MUC16

Inner mucus layer:  
attached and organized  
Outer mucus layer:  
unattached and mobile

Stomach

*MUC5AC, MUC6,*  
MUC1

Outer mucus layer:  
unattached and discontinuous

Small intestine

*MUC2, MUC6, MUC1,*  
MUC3, MUC4,  
MUC12, MUC13,  
MUC17

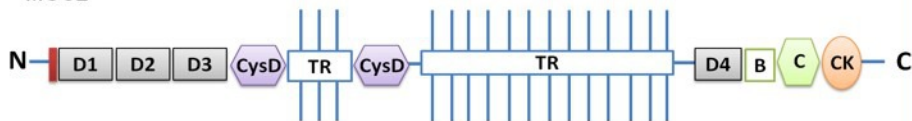
Inner mucus layer:  
attached and organized  
Outer mucus layer:  
unattached and mobile

Large intestine

*MUC2, MUC1, MUC3,*  
MUC4, MUC12,  
MUC13, MUC17

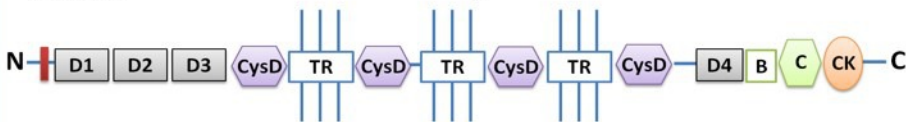
a) Secreted mucins: Gel-forming mucin

MUC2



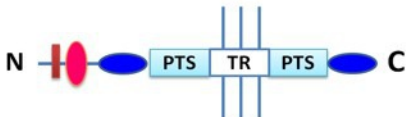
O-glycosylation

MUC5AC



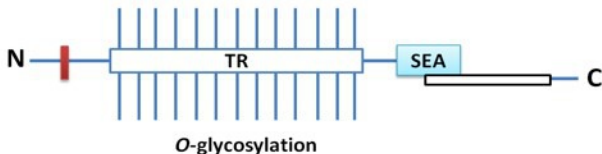
b) Secreted mucins: Non gel-forming mucin

MUC7



c) Cell membrane bound mucin

MUC1



Peptide signal

Von Willebrand D1-D4

Cysteine-rich domain

Tandem repeat (TR) sequence

Histidin like-sequence

Serine-Threonine rich region

Von Willebrand B

Von Willebrand C

Cysteine knot

SEA SEA domain

Cytoplasmic domain

Leucine-zipper

